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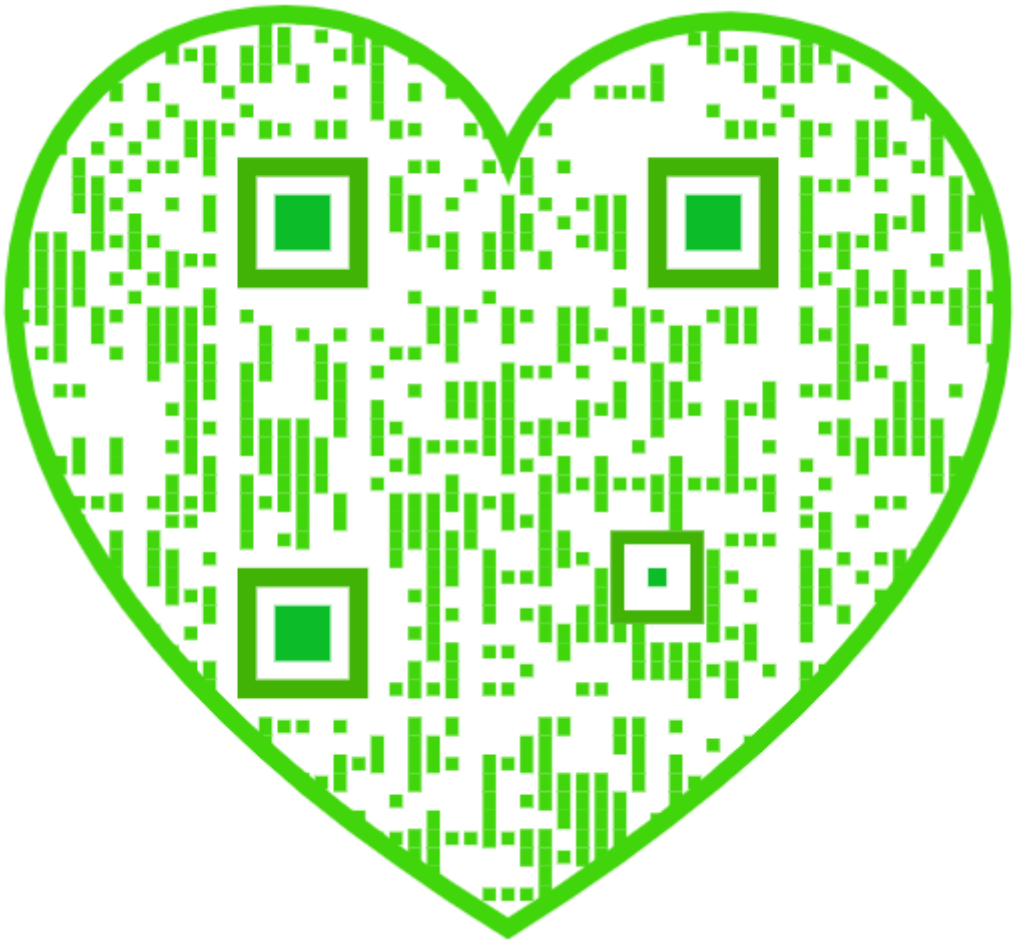

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A MANUAL

OF

CLINICAL DIAGNOSIS

BY MEANS OF LABORATORY METHODS

FOR

STUDENTS, HOSPITAL PHYSICIANS AND PRACTITIONERS

BY

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NINTH EDITION, ENLARGED AND THOROUGHLY REVISED

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TO

MY WIFE

WHO HAS SO FAITHFULLY AIDED IN ITS PREPARATION

THIS EDITION ALSO

IS

AFFECTIONATELY DEDICATED

PREFACE TO THE NINTH EDITION.

In preparing the present edition of the Diagnosis the author has carefully revised the entire work; many sections have been altogether rewritten; much new subject matter has been introduced, and last, but not least, a large number of new illustrations—many of them colored, have been added, so that the student of this rapidly growing subject will, it is hoped, find this edition also the same trustworthy guide in his daily work as the many editions that have gone before. Among the changes that have been made the writer would refer particularly to the detailed description of the ancestral types of the various leukocytes and the corresponding colored plates; the sections dealing with the intestinal animal parasites and the plate picturing the ova of the most important ones; the account of the various new methods employed in the diagnosis of acidosis; the examination of the blood for transfusion purposes; the colloidal gold reaction of Lange, etc.

The division of the subject matter into two parts—the first dealing with technical questions and the second with the collective presentation of the laboratory findings in the various diseases under their corresponding headings, which was first introduced in the seventh edition, and which the profession has found so advantageous—has been followed also in the preparation of the present edition. In the department of Clinical Pathology at the University of Maryland and College of Physicians and Surgeons of Baltimore the student during his third year is instructed especially in the technical side of the work, while in his fourth year, which is spent partly in the wards and partly in the laboratory, he is taught how to interpret the various laboratory findings. This division of the work the author would recommend to other teachers of the subject also, and he trusts that in presenting the subject matter to their students in this manner they may find the volume as useful as it was the writer's intention to make it.

Charles E. Simon.

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PART I

GENERAL PRINCIPLES AND TECHNIQUE

CHAPTER I

THE BLOOD

THE MORPHOLOGICAL ELEMENTS OF THE BLOOD

THE RED CORPUSCLES

General Characteristics. — Variations in Size and Form. — The normal red cells of the blood are greenish-yellow, circular little bodies, which in post-embryonic life are non-nucleated. According to Weidenreich and others, they are bell-shaped and not biconcave, as was formerly supposed. Their diameter normally averages 7.5 μ, with variations from 6 to 9μ. Such cells are usually spoken of as *normocytes,* in contradistinction to abnormally small or abnormally large cells, which may be met with under pathological conditions and which are called *microcytes* or *macrocytes* respectively. The term *microcytosis* or *microcythemia* is used to designate a predominance of microcytes, while *macrocytosis* or *macrocythemia* indicates a preponderance of macrocytes. Microcytes measure from 3.5 to 6μ, and macrocytes from 9.5 to 12μ in diameter; still larger cells are spoken of as *gigantocytes;* these may attain a diameter of 16μ.

As regards the origin of the macrocytes, there is evidence to show that they may develop from the common normocytes, in the circulating blood through imbibition of water, so that their occurrence from this point of view could be regarded as a degenerative phenomenon. But, on the other hand, their presence may be interpreted as evidence of a regenerative process, bearing in mind that in the bone-marrow the size of the erythroblast is apt to be larger than that of the common normocyte; such macrocytes would represent young normocytes which have prematurely entered the circulation. The microcytes probably result in part from the normocytes in the circulating blood through loss of water, but it is conceivable that some of them may also arise from macrocytes as a result of plasmolysis (schistocytosis).

In wet mounts microcytes may be seen in large numbers, if undue pressure has been exercised in the preparation of the specimen; the condition then is, of course, to be viewed as an artefact.

Microcytosis is, on the whole, of comparatively little clinical interest, and may be observed in any severe anemia. Macrocytosis is more important. To a certain extent it is seen in severe forms of anemia of whatever origin, but it is noteworthy that the presence of macrocytes in large numbers is essentially observed in pernicious anemia. During the active period of the disease the macrocytes may here represent 70 per cent. of all red cells (Lazarus).

Going hand in hand with pathological variations in the size of the red corpuscles—*anisocytosis*—there may be variations in form which may affect not only the microcytes and macrocytes, but also the corpuscles of normal size. Cells may thus be seen which resemble a flask, a kidney, a biscuit, a boat, a balloon, a dumb-bell, or an anvil, while others are altogether irregular in appearance.

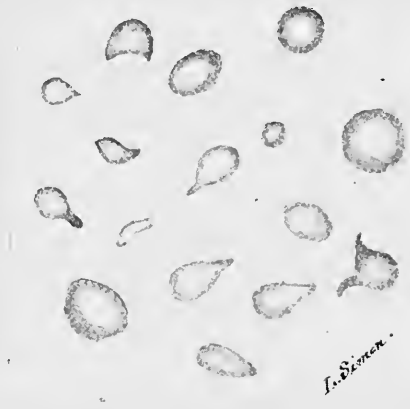
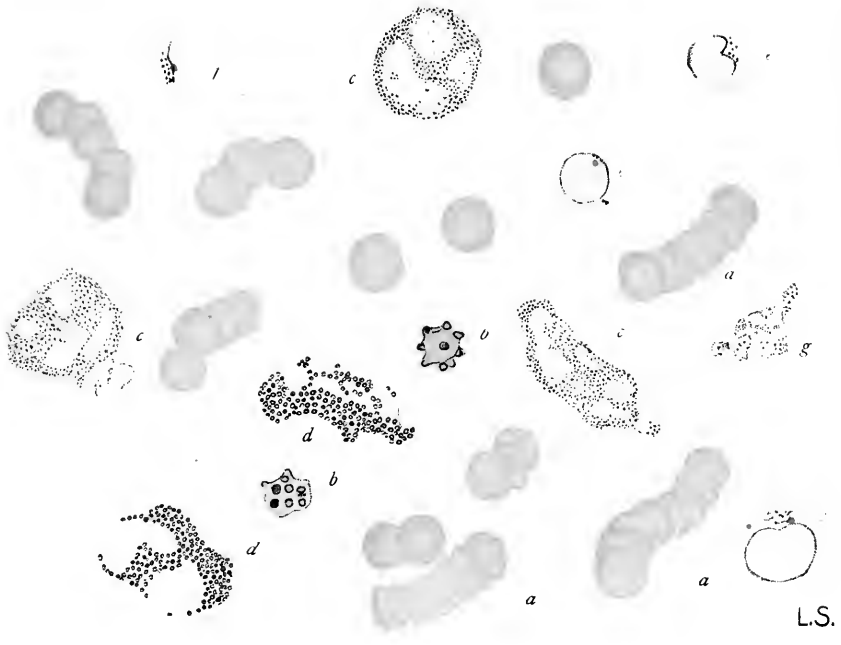


Fig. 1.—Poikilocytosis and anisocytosis.

Especially interesting is the fact that such abnormally shaped cells, which are generally spoken of as *poikilocytes* (Fig. 1), may manifest a certain degree of motility, so that they have at times been mistaken for microparasites; this is most noticeable in the smaller forms. In pernicious anemia *poikilocytosis* is most pronounced, and at one time it was thought that the condition was characteristic of the disease. It has been shown, however, that it occurs in other anemias as well, though its existence is probably always evidence of a specially severe form. In chlorosis it is usually only seen in the most severe cases, and particularly in those manifesting a tendency to thrombosis and embolism.

PLATE I



The Elements of Normal Blood.

a, red cells in rouleaux; *b*, crenated red cells; *c*, finely granular (neutrophilic) leukocytes; *d*, coarsely granular (eosinophilic) leukocytes; *e*, small, and *f*, large mononuclear leukccytes; *g*, plaques.

In this connection a special deviation from the normal form of the red corpuscles also requires consideration, viz., the prevalence of oval cells. These are notably observed in pernicious anemia and seem to be of some diagnostic importance when present in predominating numbers. They are found not only during the active periods of the disease, but frequently also in the interval between exacerbations.

Poikilocytosis is a degenerative phenomenon, and it is essential not to confound true poikilocytes with certain abnormal forms, which may be seen in any preparation and which are the result of mechanical injury, mutual compression, etc., and can readily be distinguished with practice.

In wet preparations red cells will be seen near the margin of the drop where evaporation is actively going on, which present little knobs or spicules on their surface and along the periphery. Such cells are spoken of as crenated cells. The phenomenon in itself is normal, but it is noteworthy that *crenation* may at times be observed in the centre of a carefully prepared specimen after a few seconds, while, as a rule, from fifteen to thirty minutes may elapse before the process begins to attack cells in this location. The significance of this early crenation is not known. This is also true of delayed *money-roll formation*, which is observed in various diseases, whereas normally the red corpuscles almost immediately tend to run together in rolls, unless special pains are taken to secure their separation (Plate I).

Variations in the Color of the Red Corpuscles.—The degree of coloring of the red corpuscles depends upon the amount of hemoglobin. The centres of the cells in well-mounted specimens are always paler than the periphery, and any deficiency in the amount of coloring matter is here at once apparent. With a moderate grade of anemia the cell as a whole looks paler, and the pale central area is increased in size. With a further increase in the loss of coloring matter the central area is absolutely colorless and encroaches upon the peripheral colored zone more and more until finally the so-called *pessary forms* result, in which only a narrow rim of hemoglobin remains. These changes can be made out in wet preparations, but are especially well seen in stained specimens. The central pale area is, however, visible only in well-preserved cells and not in flattened-out cells, which are stained uniformly throughout and which may also be seen in any specimen.

An increased amount of coloring matter is relatively rare, but constitutes one of the most constant symptoms of pernicious anemia.

A deficiency in coloring matter on the part of a red cell is spoken of as *hypocytochromia*, while the presence of an increased amount is termed *hypercytochromia*. In this connection it is important to bear in mind that while hypocytochromia, viz., anemia of the indi-

vidual cell, is commonly associated with a generalized oligochromemia, the latter may be accompanied by hypercytochromia. (See Color Index.)

The reasons underlying the hypercytochromia of pernicious anemia are not known. It has been suggested that this might be due to the existence in the cells of a hemoglobin, richer in hematin, than the normal, or to a degenerative change of the oxyhemoglobin with consequent increased affinity for acid dyes. Regarding the latter possibility, Pappenheim has suggested that the formation of methemoglobin would carry with it an increased affinity for acid dyes and lend a darker color to the cell.

Of the origin of hypocytochromia nothing is known that is at all definite. We speak of toxic influences, but we do not know in what manner these bring about a loss in hemoglobin, or how they prevent the formation of a normal quantity, as the case may be.

The color of the normal red cells in wet specimens is a pale greenish yellow. In malaria curiously discolored corpuscles are seen, which present a bronzed appearance; their presence should always excite suspicion. The meaning of the discoloration is not known, but in all probability it is evidence of a degenerative process.

The Color Index.—The term color index is used to designate the relative amount of hemoglobin which is contained in each corpuscle. It is determined by dividing the percentage of blood-coloring matter by the percentage of red cells as compared with the recognized normal, viz., 5,000,000.

EXAMPLE.—The percentage of hemoglobin is 50, the red count per c.mm. is 2,000,000, viz., 40 per cent. of the recognized normal, 5,000,000. The color index is then 50 divided by 40—*i. e.*, 1.25.

Under normal conditions the color index is about 1, but may vary from 0.95 to 1.17; it is slightly higher in men than in women. An increase is notably seen in pernicious anemia, while in chlorosis a low value is almost invariable. In the secondary anemias the index is either normal or, what is more common, slightly diminished. We accordingly speak of "secondary anemia of the chlorotic type."

Variations in Number.—The number of red corpuscles in the blood of healthy adults is fairly constant. In man 5,000,000 per c.mm. may be considered a fair average, and in women 4,500,000. Higher values are not uncommon, but the number rarely exceeds 6,000,000 in perfectly normal individuals.

The largest number found on the first day after birth averages 6,985,428. It diminishes until the third day. Following a temporary rise it drops further and becomes fairly constant between the sixth and the tenth day.

In 20 healthy infants Karnizki obtained the following values:

Age.	
2 to 4 months	5,239,725
4 to 8 "	5,703,000 to 5,843,000
8 to 12 "	5,531,000 to 5,590,521

After the sixth year the number is on an average higher during childhood than in babyhood.

A somewhat higher average is found among people living at a considerable elevation above the sea level, and it is interesting to note that an increase in the number occurs whenever a change in the habitation is made from a lower to a higher level. This increase is frequently quite marked, as is apparent from the following table, which is taken from Ehrlich:

Altitude.	Increase of
561 meters	800,000
700 "	1,000,000
1800 "	2,000,000
4392 "	3,000,000

A corresponding diminution occurs when a change is made from a higher to a lower level.

In this connection Gaule's observations are of interest. On the occasion of a balloon ascension to a height of from 4200 to 4700 meters he counted 7,040,000, 8,800,000, and 7,480,000 respectively in the three participants of the journey. The hemoglobin was at the same time diminished, and he accordingly concluded that the increase during the ascent was due to an increased production of red cells; the probable nature of this conclusion was strengthened by the fact that numerous normoblasts were found in the blood, many undergoing division. Jolly, Bensaude, and others, on similar expeditions, were unable, however, to demonstrate the presence of nucleated red cells or to note the occurrence of an increased number of red cells. According to Weinzirl, the increased counts due to high altitude are temporary and in part at least referable to cold. He showed that in rabbits a certain increase in the number of red cells occurs when they are removed from warm to cold quarters, and that their subsequent removal to a higher altitude does not lead to a further increase.

In disease the number of the red cells may be either increased or diminished. The term *polycythemia* or *polyglobulism* is used to designate the first, and the term *oligocythemia* the latter condition. Clinically, we distinguish between relative and absolute polycythemia. *Relative polycythemia* is much the more common and usually due to a concentration of the corpuscular elements owing to a loss of fluid from the body. It is thus frequently seen when there has been much sweating, diarrhea, or vomiting, but it is also common in cases where great tissue waste is going on and where water is either lost through other channels, or where adequate storage of water does not take place. The ordinary wasting diseases are common examples of this type. Blood examination in such cases frequently furnishes no idea whatever of the extreme grade of anemia which actually exists, and a proper insight into the actual condition would only be possible if we could estimate the amount of blood as a whole and make

appropriate correction for the amount of fluid that is lacking. Satisfactory methods for this purpose are unfortunately not available.

More rarely relative polycythemia is due to vasomotor disturbances; this is noted in poisoning by phosphorus, carbon monoxide (up to 11,200,000), and various coal-tar products, during and immediately after the administration of ether, following cold baths, severe muscular exercise, etc. Of similar origin probably is the polycythemia which is noted in disease of the adrenal glands, where counts of from 6,000,000 to 7,000,000 have been repeatedly noted; and the same is probably true of diabetes, in which polycythemia may be observed both while fasting and while much fluid is being ingested.

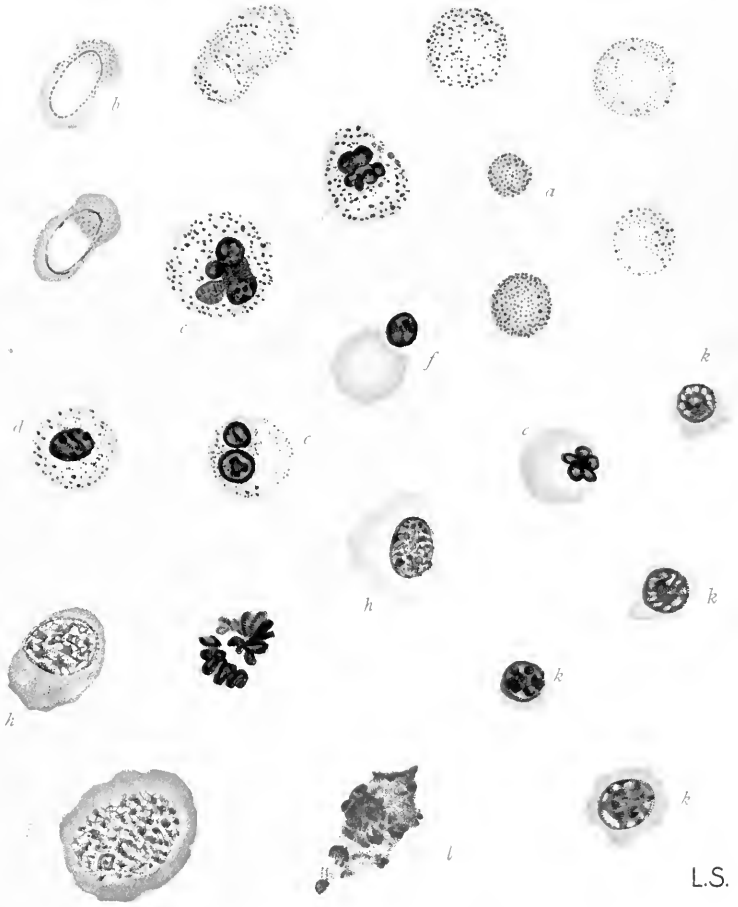
Absolute polycythemia is far less common than the relative form. It is essentially encountered in conditions in which there is persistent difficulty in the proper aëration of the blood and must be viewed as a vicarious attempt on the part of the body to overcome such deficiency It is notably seen in congenital heart disease, where the figures commonly reach 8,000,000 to 9,000,000; less markedly, as a rule, in acquired heart disease, and most pronounced in Osler's disease. (autotoxic enterogenous cyanosis, erythremia) (up to 12,000,000).

While there can thus be no doubt that a true polycythemia does occur, it has been conclusively demonstrated that such a condition does not exist in what is generally termed *plethora*, and that the various symptoms of plethora formerly attributed to a general increase in the amount of blood are referable to vasomotor disturbances.

Oligocythemia is much more common than polycythemia. It may be temporary or permanent, and is seen in all forms of anemia of whatever origin. The lowest counts are met with in pernicious anemia, in acute streptococcus infections, particularly of puerperal origin, and in malaria. The lowest reported count was made by Osler, in a case of pernicious anemia, shortly before death—100,000. Corpuscular anemia of the secondary type, when occurring in wasting diseases, is usually more or less obscured by the associated relative polycythemia, as has just been pointed out.

Behavior of Red Corpuscles toward Anilin Dyes.—**Polychromatophilia** (**Polychromasia**).—The normal *living* red cell possesses no affinity for dyes; it is achromatophilic. The normal *fixed* cell of the circulating blood, on the other hand, has a marked affinity for acid dyes, such as eosin, orange-G, acid fuchsin, etc.; it is accordingly said to be oxyphilic, and as it takes up only one color from a mixture of different dyes it is termed monochromatophilic. Under various pathological conditions which are associated with a marked grade of anemia cells are met with which are polychromatophilic. Such cells manifest an affinity not only for acid dyes, but simultaneously also for basic dyes, so that with a mixture of eosin and methylene blue, for example, the red cells are not stained in the usual tint of the hemoglobin, but present a mixed color in which that of the basic dye is more or less apparent (Plate II).

PLATE II



a, a group of red cells undergoing granular degeneration (stipple cells); *b*, red cells, showing Cabot's ring bodies; *c*, normoblasts with nuclei undergoing karyolysis; the bodies of the cells show granular degeneration; *d*, normoblast with pyknotic nucleus; *f*, red cell, suggesting loss of nucleus by extrusion; *g*, red cell undergoing mitosis; *h*, megaloblasts with polychromasia of protoplasm; *i*, gigantoblast; *k*, young normoblasts, showing spoke-like arrangement of the chromatin; *l*, a group of plaques.

As regards the significance of the polychromasia, Ehrlich maintained that the condition is evidence of a degenerative process—of a coagulation necrosis of the discoplasm as a consequence of which this takes up albumins from the blood plasma, while it loses the power of holding its hemoglobin. The oxyphilia hence diminishes, while owing to the absorption of albumins a more or less pronounced basophilia develops. As a matter of fact, polychromatophilia is often seen in cells which are manifestly degenerating, and Ehrlich accordingly speaks of it as *anemic or polychromatophilic degeneration* of the blood. There is a growing tendency, however, at the present time to view polychromatophilia essentially as evidence of youthfulness or immaturity on the part of a cell, while admitting, of course, that a young cell may undergo degeneration as well as a mature cell. As a matter of fact, we find that the erythroblasts of the normal bone marrow are for the most part polychromatophilic, and the more markedly so the younger they are (Plate II). In those animals, moreover, in which the red cells of the circulating blood are normally nucleated a certain grade of polychromasia, according to my experience, appears to be the rule in all the younger cells, while the pure hemoglobin tint is only obtained in the mature forms.

While in certain animals, such as the guinea-pig, mice, dogs, cats and others polychromatophilic red cells are commonly met with in the absence of any symptoms of disease, such an occurrence is infrequent in the human being, but may at times be observed in children or young adults.

It is well to bear in mind that polychromasia in itself is not a symptom of anemia. Its existence merely indicates a certain degree of blood destruction which may or may not be balanced by a corresponding blood regeneration.

Diabetic Chromatophilia.—Bremer has pointed out that a difference exists in the affinity of a diabetic blood for certain anilin dyes, as compared with non-diabetic blood. For, whereas non-diabetic blood is readily stained with Congo red, methyl blue, eosin, etc., diabetic blood is distinctly refractory, while such dyes as Biebrich scarlet, which readily stain the diabetic blood, do not color non-diabetic blood.

Regarding the nature of the substance in diabetic blood which is responsible for this peculiar behavior little is known, but it appears certain that the reaction is not dependent upon the presence of glucose nor upon the degree of alkalinity of the blood, as suggested by Lépine and Lyonnet.

Granular Degeneration of the Red Cells.—Under certain pathological conditions red cells may be met with which contain basophilic granules. These are readily stained with methylene blue, methylene azure, thionin, etc. Methyl green, however, which is a specific

nuclear dye, does not stain the granules. Their size, form, and number are variable. While the majority are round, others are rod-shaped or biscuit-shaped. The largest granules are found in pernicious anemia and in cases of lead poisoning with intestinal manifestations. They are then quite readily seen and attract attention at once (Plate II). In most other diseases in which they occur they are much smaller, and on superficial examination they may indeed be overlooked; some cells at first sight merely look a little off-color, and it is seen only on very careful examination that the apparent polychromasia is in reality due to the presence of large numbers of minute dots. Very often, in anemic cells, the granules are arranged in the peripheral portion of the cell, lying in the zone occupied by the hemoglobin. Their number is exceedingly variable; generally speaking, it depends upon their size; when they are especially large they are relatively less numerous; when minute the cell appears as though dusted over with them.

The granules may occur in cells of normal size and color, in poikilocytes, and in nucleated red cells, both of the normoblastic and the megaloblastic type, especially the former. Not infrequently they are seen in cells which are markedly polychromatic, but, like Grawitz, I do not believe that granular degeneration represents a phase of polychromasia, as Pappenheim maintains.

In disease they are most constant and numerous in pernicious anemia, in lead poisoning, and in malaria; they are less constant and less numerous in the leukemias, in pseudoleukemia, in the cachexias referable to septic infection, syphilis, carcinomatosis, and in the final stages of tuberculosis. In chlorosis and in the anemia of chronic nephritis they are absent; in two cases of v. Jaksch's anemia, in which nucleated red cells were quite numerous, I obtained negative results. The question, whether they ever occur in the blood of normal individuals I would now reluctantly answer in the affirmative; this, however, is unquestionably very rare.

As regards the significance of the granules, Engel, Ehrlich, and others have suggested that they are most likely products of karyorrhexis. Others maintain, and I think rightly so, that they are not of nuclear origin. They may be found at a time when not a single nucleated red cell is demonstrable in the blood and nucleated red cells may be seen in which no sign of karyorrhexis is manifest, while the body of the cell is studded with granules. They may be found in nucleated cells which are undergoing karyokinetic division. Unlike the nuclei of the erythroblasts, the granules have no affinity for methyl green, which is a specific nuclear dye. This can be shown very well by staining with methyl-green-pyronin, when granular products derived from nuclei are stained green, while the stippling in the same cell appears red. A few observers claim to have stained

the granules with methyl green; this merely shows that their dyes were contaminated with methylene blue.

According to Grawitz and others granule cells are not commonly found in the bone marrow even when they are numerous in the circulating blood; when they do occur, they are not more numerous than in the peripheral vessels. Grawitz hence regards their presence as an indication of a degenerative change in the hemoglobin, and speaks of the phenomenon as "granular degeneration." Others regard the bone marrow as their place of formation.

According to Pappenheim stippling is not found in erythroblasts in the bone marrow under normal conditions, but only when there is excessive regeneration, as in the embryo, in pernicious hemolytic anemia, in myelophthisic neoplastic anemia, in myelogenous pseudoleukemia and lymphadenoid leukemia and lymphosarcomatosis of the bone marrow. Schmauch has observed similar appearances in the blood of healthy cats, and Engel has described the occurrence of granule cells in the blood of early cat embryos. I have found granule cells in the blood of various animals, and, as I have said before, occasionally one meets with an isolated cell in apparently normal individuals.

Whether or not the granule cells of Vaughan, which this observer has demonstrated in normal wet specimens with Unna's polychrome methylene blue are identical with the variety described above is not certain. Their number varied quite constantly between 1.8 and 5 per cent. The examinations were conducted with wet blood, a drop of the staining fluid being placed upon the site of the puncture. At first the granules are red, but after some time they change through a purple to a pronounced bluish. Positive results were also obtained under various pathological conditions, especially in pernicious anemia, where their number was about ten times as great as in normal blood. In newborn infants they averaged 4.7 per cent. Analogous results have been obtained by Cadwalader. Vaughan regards the granules as nuclear remains, and states that he rarely found this type of stippling and nuclei in the same cell.

Not to be confounded with "granular degeneration" is the stippling of Schüffner, Ruge, and Goldhorn, which is seen in red cells infected with tertian parasites (Plate XII, *c'*). This is brought out with methylene azure and may hide the parasite from view. It is viewed as a degenerative phenomenon.

Cabot's Ring Bodies.—Cabot has drawn attention to the occasional occurrence in red cells of curious ring bodies which are usually stained red with Wright's modification of Leishman's stain, but which may also take on a blue color. He found such rings in pernicious anemia, in lead poisoning, and in lymphatic leukemia. I have been able to demonstrate the same structures with the eosinate of methylene blue, and could verify Cabot's observation that they occur in granule

cells, but may also be found in apparently normal red corpuscles (Plate II and Fig. 2). No doubt they bear some relation to the nucleoids.

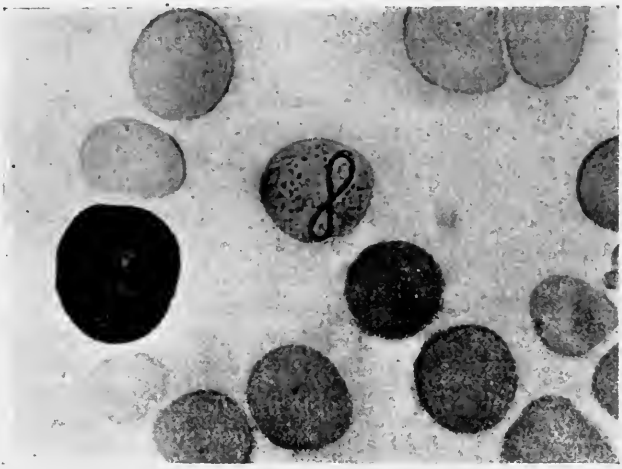


Fig. 2.—Cabot's ring bodies.

Ehrlich's Hemoglobinemic Inner Body (Innenkörper).—These structures may be encountered in red cells in conditions associated with extensive hemocytolysis the result of specific blood poisons (nitrobenzol and certain anilin derivatives). The individual body is round and characterized by an increased affinity for acid dyes, so that it appears in the stained specimen as a deeper staining mass lying embedded in the red cell, but presenting the same color as the latter. Frequently, also, it is seen in process of extrusion from the cell. These formations are now viewed as local accumulations of methemoglobin, the outcome of the toxic action of the poison in question upon the oxyhemoglobin.

Nucleated Red Corpuscles.—The Erythroblasts.—Nucleated red corpuscles are not found in the circulating blood of normal individuals, excepting at birth and during the first days of life, when it is not unusual to meet with an occasional cell of this type. In the bone-marrow, however, they are always found. It is here possible to distinguish two types, viz., the normoblast and megaloblast. The latter is ontogenetically the older and gives rise to the normoblast through a process of heteroplastic differentiation following cell division; it thus bears the same relation to the normoblast which exists between the large lymphocyte and the small lymphocyte, and the amblychromatic myelocyte and the trachychromatic myelocyte (which see). The megaloblast in turn results from the large lymphocyte (lymph-

oidocyte) through heteroplastic transformation and ages into the macrocyte, while the normoblast similarly develops into the normocyte. (See Fig. 3.)

While at a certain period of embryonic life megaloblastic blood corpuscle formation plays a prominent role, megaloblasts are found only in small numbers in the bone marrow of the normal adult. Normoblasts, on the other hand, are numerous and control the usual red corpuscle production exclusively.

The Normoblasts.—The normoblasts (see Plate II), like the normal red cells of the circulating blood, have a diameter which varies from 6 to 9μ. The nucleus in the youngest cells occupies a central position, and is larger and relatively poorer in chromatin than in the older cells, where it is frequently located eccentrically. The size varies between 2 and 4μ. The appearance of the normoblast in the peripheral circulation is variable (Plate II). In most cases young cells are seen with a radiary arrangement of the chromatin and polychromatophilic protoplasm. At other times older cells with densely staining pyknotic nuclei and oxyphilic protoplasm are encountered, and again we may meet with cells in which manifest karyolysis is going on. Fragmentation of the nucleus (karyorrhexis) may likewise be seen, as also free nuclei as such. The so-called Howell, Schmauch-Jolly bodies, which may also be encountered, are merely single nuclear remnants, representing an intermediary stage between the pyknotic condition of the nucleus and its disappearance through chromatolysis. Mitoses are not uncommon in pernicious anemia and leukemia.

In the majority of cases in which normoblasts are found in the blood they are well developed, but in myeloid myelocytic leukemia more especially it is common to meet with cells in which the protoplasm surrounding the nucleus is reduced to a little hood which is apparently attached to one side of the nucleus (Plate II). Such cells in my experience are always polychromatophilic and are apt to be mistaken by the beginner for lymphocytes. They probably represent immature cells.

The occurrence of normoblasts in the circulating blood is always evidence of stimulation of the bone marrow, which may occur either indirectly, as the result of an "anemic" condition of the blood (secondary myelopathy), or directly, as in disease of the bone-marrow *per se* (primary myelopathy). We may accordingly meet with normoblasts in almost any form of anemia, be this the result of traumatism (posthemorrhagic), of inanition, or of organic disease.

The number is quite variable. In the ordinary types of secondary anemia they are usually rather scarce. They are most numerous in acute cases. In pernicious anemia, and especially in the myelocytic type of leukemia, they are frequently present in considerable numbers. In the first-mentioned disease, their continued absence is usually

evidence of an aplastic condition of the bone-marrow and hence of bad omen.

At times there occur sudden invasions of the circulating blood by red cells, many of which are nucleated; this phenomenon v. Noorden terms a *blood crisis*, and it is noteworthy that the invasion of the red cells may be preceded and accompanied by a very extensive increase of the leukocytes.

Blood crises are seen most frequently in pernicious anemia, but are occasionally encountered in symptomatic anemias as well. Through the kindness of Dr. D. Moore, of the Marine Hospital Service, I saw an extreme instance of this kind in a case of cancer of the right kidney, where 175 normoblasts were seen while counting 200 leukocytes. I do not recall the total number of the leukocytes, but remember that they were not diminished.

In cases of traumatic anemia unusually small nucleated red cells have at times been observed. These are termed *microblasts*. They have attracted but little attention and are quite rare. I have seen such cells, measuring not more than 3 to 3.5 μ, in a case of pernicious anemia at the time of a blood crisis, when large numbers of normoblasts were also present.

The Megaloblasts.—These are usually from two to three times as large as the normoblasts, and may attain even more extensive proportions (Ehrlich's gigantoblasts). (See Plate II.) But some specimens are only a very little if at all larger than the common red cells; these probably represent young daughter cells. The megaloblasts are provided with a relatively large centrally located nucleus, which is wide-meshed and which with the triacid stain is not colored nearly so deeply as the normoblastic nucleus. In some specimens, indeed, the affinity for methyl green is so little marked that at first sight a nucleus can hardly be distinguished. With those staining mixtures, on the other hand, which contain methylene blue as base, it can always be fairly well made out. But owing to the fact that these cells are usually polychromatophilic, the nucleus may at first be overlooked, as the polychromatic protoplasm appears in the meshes of the nucleus and sometimes differs but little in color from the chromatin. The inexperienced not infrequently mistake such cells for large mononuclear leukocytes that are somewhat off-color; the character of the nucleus, however, viz., its wide meshwork, should prevent this mistake. Pyknotic megaloblasts do not exist, so that the presence of a pyknotic nucleus in an erythroblast of doubtful nature indicates its normoblastic type.

Mitoses in megaloblasts are rarely seen.

The amount of protoplasm is variable, relatively small in the young cells and more abundant in the old ones.

The deciding factor in the diagnosis of the megaloblast is *not the size of the cell*, but the structure of the nucleus.

As already mentioned, the megaloblast is essentially a cell of embryonic life. After birth, under normal conditions, a few megaloblasts may be found in the blood of very young infants, and it is noteworthy that in the severe types of secondary anemia megaloblasts are far more apt to occur in children than in adults. But even then they are rare. In the bone marrow of the adult they are present in very small numbers. According to Ehrlich, the presence of megaloblasts in the blood is evidence of a reversion of the blood formation to the embryonic type and of grave prognostic import. He once regarded their presence as indicative of essential pernicious anemia; and, as a matter of fact, they are here quite constantly met with and represent one of the most important features of the disease. They are rarely numerous, however, and there are cases in which they are absent (aplastic anemia).

The modern tendency is to regard the appearance of megaloblasts in the blood as evidence of an anemia of unusual severity, viz., as a degenerative-regenerative symptom, and not as an indication of any one disease. While they are undoubtedly most constant in pernicious anemia, they may also be met with in other forms. They have been found in leukemia, in the pseudoleukemia of infants, in lead poisoning, and even in chlorosis, and, as I have pointed out already, in some of the severe types of secondary anemia occurring in young children. In cancer of the stomach, according to Osler and McCrae, they are rarely if every found. Askanazy has reported an interesting case of bothriocephalus infection in which the megaloblastic type of blood regeneration disappeared after expulsion of the parasites—sixty-seven in number—and was replaced by the normoblastic type, the case ending in recovery.

The appearance of megaloblasts in extra-uterine life merely indicates an incomplete maturation of young elements, their consumption and consequent increased production. The following sketch, taken from Pappenheim, gives an idea of the relation of normoblasts and megaloblasts to the different types of anemia:

Under normal conditions Pappenheim's large lymphocyte (lymphoidocyte) (see Fig. 3) gives rise to the young megaloblast, which in turn differentiates itself into the young normoblast. The young normoblast ages to the pyknotic normoblast and loses its basophilic nuclein as a result of chemical karyolysis. In this manner an apparently non-nucleated erythrocyte results, which loses its nucleoid later, in the blood, as blood platelet in consequence of variations in the tonicity of the plasma. In severe toxogenic anemias, on the other hand, there is an arrest of development upon an embryonic basis. A certain proportion of young megaloblasts multiplies homoplastically; another portion matures to old megaloblasts, while a third fraction only becomes differentiated to young normoblasts. Of these in turn one portion matures to the old forms, which dislodge their nuclei in the anemic

serum in toto, while another portion loses the nucleus during the process of hastened maturation by karyorrhexis. As a consequence, many of the anemic normocytes contain no nucleoids, and the blood as a consequence contains only small numbers of blood platelets.

Pyknotic normoblasts, as also young megaloblasts (of the type of the large lymphocyte), may be encountered in all forms of severe anemia of whatever origin. In the kryptogenetic type of pernicious anemia and bothriocephalus anemia, however, *old* megaloblasts (of the type of the large mononuclear leukocyte) are further seen, as also young normoblasts (of the type of the small lymphocyte) undergoing karyorrhexis.

Generally speaking, the number of erythroblasts is no indication of the severity of the case, but merely indicates the extent to which the bone marrow responds to the blood destruction. The appearance of megaloblasts is hence not necessarily an unfavorable symptom, but simply the expression of an unusually high activity of the erythropoietic tissue.

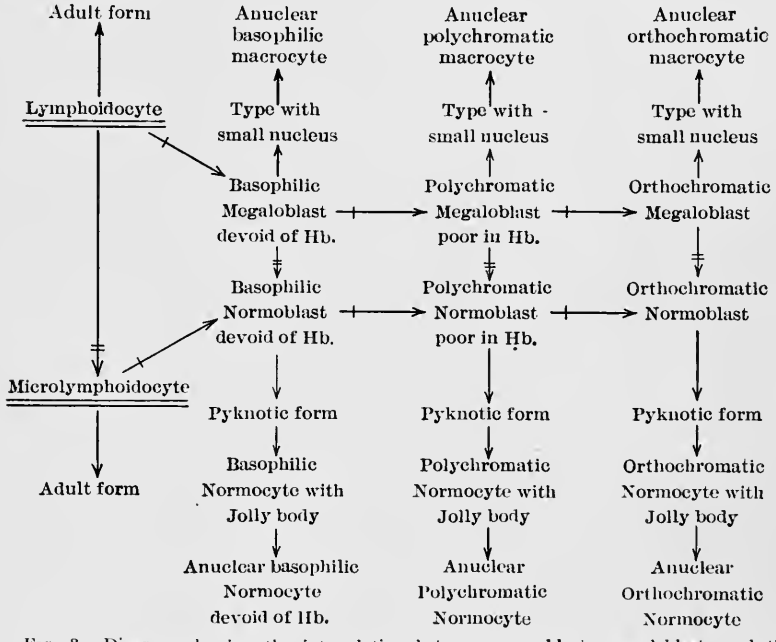


FIG. 3.—Diagram showing the interrelation between normoblasts, megaloblasts and the corresponding erythrocytes and their derivation from the aneestral lymphoidocyte. ⟶ direct aging. —|⟶ reproduction with complete differentiation. —||⟶ reproduction with incomplete differentiation. (Taken from Pappenheim.)

THE LEUKOCYTES

General Characteristics.—The leukocytes, or white corpuscles of the blood, as seen in the wet preparation (Plate I), are roundish

or irregularly shaped cells, which vary in size, but for the most part are larger than the red corpuscles. They are all nucleated, and, as the term indicates, devoid of coloring matter. In a general way they may be divided into two classes, viz., those which are granular and those which are not granular.

Granular Cells.—The granular cells (granulocytes) are by far the most numerous, and are characterized by the fact that they are capable of active locomotion. Even without a warm stage it is almost always possible to observe this in the ordinary wet preparation. The moving cells at once attract attention by their irregular outline. On careful examination with a high power it will be noted that the cell advances in a definite manner, which is quite analogous to what is seen in the ameba. The protoplasmic portion manifestly consists of two parts, viz., a non-granular hyaline ectosarc and a granular endosarc. As the leukocyte progresses the hyaline ectosarc advances with a flowing motion, forming a layer in front of the granular endosarc, which itself then merges into the non-granular portion. The moving leukocyte is roughly pear-shaped, with the base in advance, while the rear end tapers markedly and frequently seems to drag behind it a small, roundish mass, which, like the main body of the cell, is granular. The nucleus of the granular leukocytes is either polymorphous—*i. e.*, it is composed of different lobes which are joined together—or it may be multiple. Such cells are hence spoken of as polymorphonuclear and polynuclear leukocytes, respectively. The polymorphous cells supposedly represent an earlier stage in the development of the polynuclear forms.

While the granules in the majority of the leukocytes are fine (Plate I), on careful search some cells will be found in which they are coarse and highly refractive, resembling tiny fat globules. This coarsely granular variety is very characteristic in appearance and at once attracts attention. The cells are far less numerous, however, and, as a matter of fact, represent only from 1 to 4 per cent. of the total number of the leukocytes, while the finely granular variety represents from 60 to 70 per cent. Like the finely granular variety, they are capable of moving about, but their phagocytic function, toward bacteria at least, is insignificant. The finely granular cells are the true *phagocytes* of Metschnikoff.

Non-granular Cells.—The non-granular leukocytes, in contradistinction to the granular variety, are mononuclear, with very little tendency to polymorphism. They are quite hyaline in appearance, and are readily overlooked by the beginner unless a somewhat subdued light is used in the examination. Two varieties may be recognized—one about the size of a red corpuscle, the other somewhat larger. The nucleus in both varieties occupies a considerable portion of the cell and is surrounded by a layer of protoplasm which is practically hyaline. Every cell, it is true, contains a few granules collected

at a certain point along the periphery, where the protoplasm is more extensively developed than elsewhere; but these granules, in contradistinction to those which we see in the polynuclear varieties, probably represent nodal points in the cytoreticulum, and not a specific secretory product, as which Ehrlich and his school view the granules of the polynuclear variety. In the small mononuclear form one or sometimes two small, brownish granules can usually be discerned somewhere in the peripheral layer of the protoplasm. Of the significance of this granule, so far as I am aware, nothing is known, nor has its presence been previously described (Plate I).

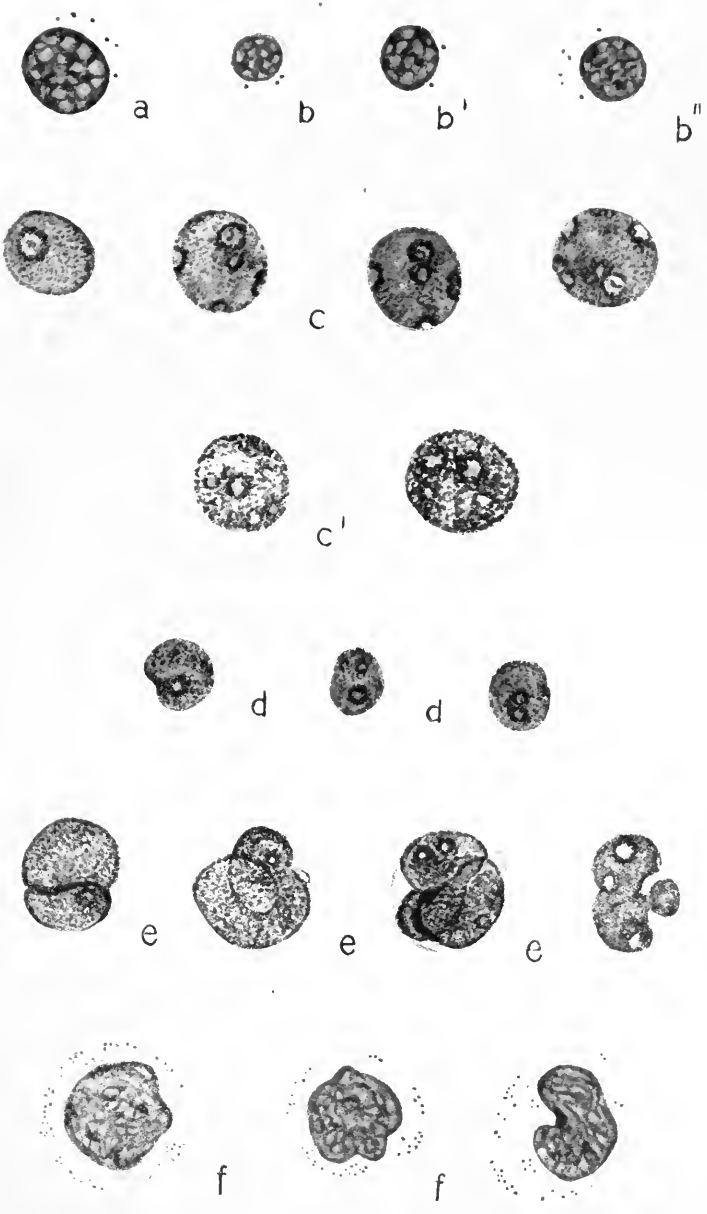
The non-granular mononuclear leukocytes, in contradistinction to the polynuclear granular variety, were formerly regarded as non-motile. Jolly, Wolff, and others have shown, however, that they also are capable of changing their form even though progressive locomotion may not occur. This can readily be demonstrated even without a warm stage, and it will be observed that the nucleus takes an active part in these changes.

Classification.—While it is possible to distinguish the different varieties of leukocytes in the wet and unstained preparation, a more complete picture of the structure of the individual forms may be obtained from the study of stained specimens.

In Normal Adult Blood We Distinguish the Following Varieties:

1. **The Lymphocytes** (*Small Mononuclear Leukocytes, or Microlymphocytes*) (Plate III).—These cells are for the most part a little smaller than the red corpuscles or of equal size. The nucleus is single and surrounded by a narrow rim of protoplasm which is generally described as non-granular. Nucleus and protoplasm are both basophilic, and, generally speaking, the protoplasm is so more markedly than the nucleus. This is best seen in specimens which have been stained with a methylene-blue mixture, where the lymphocytes for the most part present a comparatively feebly staining nucleus which is surrounded by a rim of dark blue. Other cells belonging to the same group, however, will also be seen in which this is not so marked, but in which the staining affinities of both nucleus and protoplasm appear about the same or in which the protoplasm may even be lighter in color. These cells are generally a little larger than the first variety, with a somewhat broader zone of protoplasm and an eccentric position of the nucleus. They represent a later stage in the development of the deeply staining cell, and may be classified as medium-sized lymphocytes. Still larger forms are rarely seen in the blood of adults, but may be encountered in that of infants. They are termed large lymphocytes of the normal type, and should not be confused with the so-called lymphoidocytes which are the ancestral cells from which all leukocytes are derived or which closely resemble the normal type (see below).

PLATE III



a, macrolymphoblast; *b*, microlymphocyte; *b'*, older form; *b''*, monocytoid form; *c*, macrolymphoidocytes; *c'*, monocytoid forms; *d*, microlymphoidocytes; *e*, Rieder forms of macrolymphoidocytes; *f*, large monocytes.

With certain dyes, like methylene blue, the protoplasm of the lymphocytes does not appear perfectly homogeneous, but presents a peculiar granular appearance. This is referable to nodal points in the cytoreticulum and does not represent a true granulation. With methyl green, and hence with Ehrlich's triacid stain, the protoplasm is perfectly homogeneous and appears as a pale rim surrounding the somewhat more deeply staining nucleus. While it is thus impossible with the usual dyes to demonstrate the existence of a true granulation in the lymphocytes, Michaelis has called attention to the fact that with eosin-methylene-azure solutions (see Stains) azurophilic granules can be demonstrated in some of the cells. Their significance is unknown. Very curiously these granules are not demonstrable in the lymphocytes obtained from the lymph glands directly, and it appears that they are present in only a certain percentage of those occurring in the blood. The number in a cell is variable; in some only two or three are seen, while in others the protoplasm is literally studded with them. Their size varies between that of the common neutrophilic and that of the eosinophilic varieties (Plate VII).

In wet specimens, as I have pointed out, one or two reddish-brown granules are quite commonly demonstrable in most of the lymphocytes. In stained preparations these cannot be seen.

The outline of the cell in the smaller forms is usually fairly smooth, but in the larger varieties it is often shaggy, and at times specimens are seen with a number of distinct knobs.

The nucleus, in the smaller forms especially, is concentrically located, while in the larger varieties, in which the protoplasm is more extensively developed, it commonly occupies an eccentric position. In the stained specimens, especially in the larger cells, it is sometimes surrounded by a faint areola, which is probably owing to artificial retraction. The nucleus is more commonly oval or bean-shaped than round; deep invaginations are not often seen and fragmentation of the nucleus is rare (Rieder's lymphocytes).

Lymphocytes undergoing mitosis are sometimes seen in the blood of lymphatic leukemia. Characteristic figures, however, are comparatively rare, and it is more common to meet with cells in which division of the nucleus has already occurred. In hematoxylin-eosin specimens it is usually possible to demonstrate a nucleolus, but in eosin-methylene-blue preparations my experience has been that they are not usually seen in the lymphocytes of the normal blood, and seem to be comparatively infrequent also in the blood of lymphatic leukemia. Occasionally, however, specimens are met with in which they are distinct, and sometimes multiple.

In adults the number of the lymphocytes normally varies between 20 and 30 per cent. At birth they are less numerous. During the first twenty-four hours, in fact, there is an increase of the polynuclear neutrophiles. After that the lymphocytes rapidly increase in number,

so that by the twelfth day they represent 45 per cent. of all leukocytes (Carstanjen). Gundobin gives 59 per cent. as average value for sucklings as compared with 34.6 per cent. of polynuclear neutrophiles. After the fifth year adult values are the rule. In adult life a physiological increase of the lymphocytes is notably seen in connection with the increase of the polynuclear neutrophiles which occurs during the process of digestion.

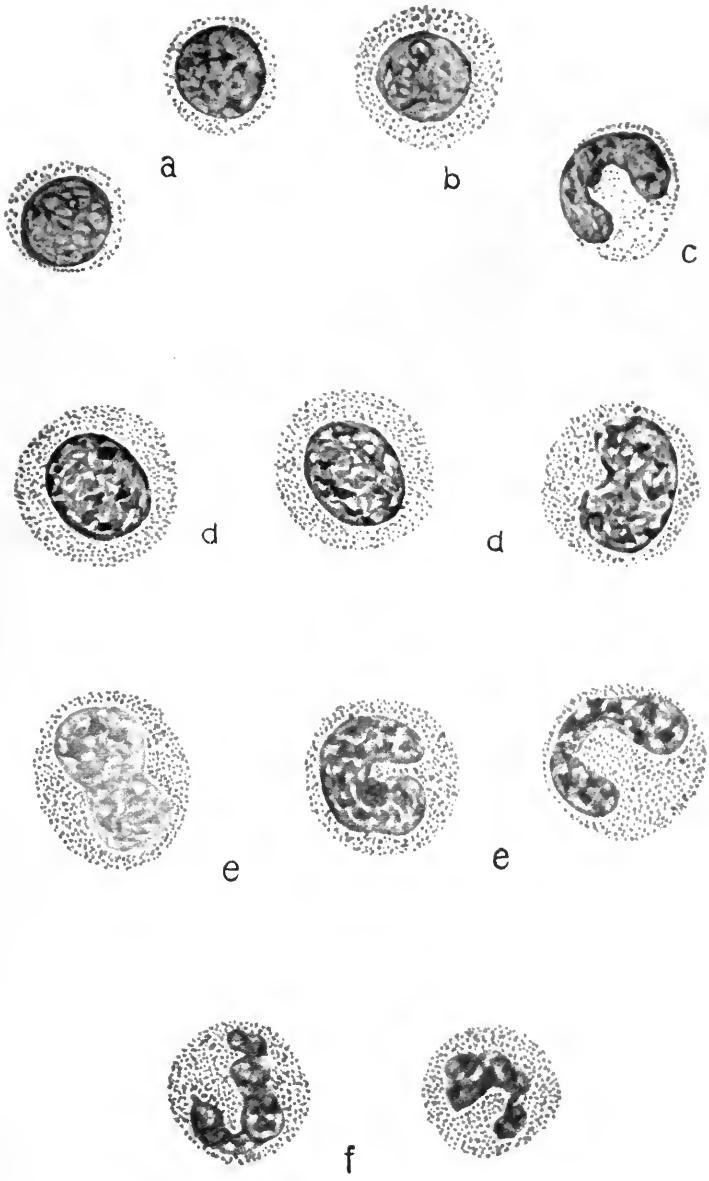
In disease the number of the lymphocytes may be increased or diminished, conditions which are spoken of respectively as *lymphocytosis* and *lymphopenia*. (See section on Leukocytosis.)

The lymphocytes of the normal blood are formed in the lymph glands, and are probably derivatives of the large lymphocytes—lymphoblasts—of the germinal centres, resulting from these through a process of differentiating reproduction. They are fully differentiated cells and incapable of further development.

2. **The Large Mononuclear Leukocytes** (*Splenocytes, Monocytes, Lympholeukocytes*).—These are mostly two or three times as large as the red corpuscles and provided with a large single nucleus, which is surrounded by a relatively wide zone of non-granular protoplasm (Plate IV). The nucleus in some cells is oval or elliptical, while in others it is more or less invaginated (Ehrlich's transition forms).

In the wet preparation the large mononuclear leukocytes are exceedingly hyaline, so that they are readily overlooked by the beginner. Both nucleus and protoplasm are basophilic, but much less markedly so than in the lymphocytes, and it is noteworthy that the protoplasm usually possesses a less marked affinity for the basic dye than the nucleus. Cells are also met with, however, in which the affinity for the dye is about the same in both. If by chance this occurs in specimens which are somewhat smaller than usual, a certain amount of difficulty arises in differentiating such small "large" mononuclear leukocytes from the older lymphocytes. A hard-and-fast line of distinction cannot be drawn, and in every differential leukocyte count the personal equation will of necessity enter into consideration. The salient characteristics of the two types should, however, be borne in mind: In the lymphocytes the protoplasm is but feebly developed in relation to the size of the nucleus, while in the large mononuclear leukocyte the reverse is true. The protoplasm in the latter, moreover, is apparently much more delicate in structure, and is readily wrinkled by contact with adjacent cells; not infrequently cells of this type are found which have manifestly been torn or otherwise injured during the preparation of the specimen; the lymphocytes, on the other hand, are usually well-preserved and clear-cut, sharply defined cells. Generally speaking, the younger forms present a darker staining protoplasm than the older ones, while the protoplasm in the latter is at the same time more extensively developed. In the oldest forms the nucleus shows a tendency to become poly-

PLATE IV



a, typical leucoblast; *b*, old form; *c*, monocytoid form; *d*, neutrophilic myelocytes; *e*, neutrophilic metamyelocytes; *f*, polymorphonuclear neutrophiles.

morphous, and it is this type which Ehrlich formerly designated as the so-called *transition form*, believing that this type changed into the polymorphonuclear neutrophilic leukocyte. This view, however, is erroneous. There is no reason for placing such cells in a special group, as many laboratory workers are still in the habit of doing. The "transitional" cells are merely old large mononuclears and should be grouped under this heading. It is most unfortunate that some writers speak of the large mononuclear leukocytes as large lymphocytes. The latter term was applied by Ehrlich to the ancestral form of all the leukocytes and should be confined to these exclusively.

In preparations that have been stained with Ehrlich's triacid both nucleus and protoplasm are very faintly colored and the latter appears perfectly homogeneous; but in specimens which have been stained with mixtures containing methylene blue as the basic component, the protoplasm presents a somewhat granular appearance, which, as in the lymphocytes, is referable to the existence of a cytoreticulum. A certain proportion of the large mononuclear leukocytes (including the transition forms), as in the case of the lymphocytes, also contains azurophilic granules.

Inclusive of the "transition forms" the large mononuclear leukocytes normally represent from 1 to 6 per cent. of the total number. They are relatively more numerous in young children, in whom the highest values are found between the sixth and ninth days after birth. Many of the cells at this time are of the type of the transition form; they may number 18 per cent.; but even in older children one commonly finds a larger proportion of these cells than in adults. An increase in the number of the large mononuclears is spoken of as "large mononucleosis" or splenocytosis.

The large mononuclear leukocytes, like the small lymphocytes, are derived from the lymphoidocyte, but it is still a question whether they represent a special group of cells or are merely aged lymphocytes of the lymphoblastic type.

For the most part the large mononuclear leukocytes develop in the spleen (hence the term splenocytes).

3. **The Neutrophilic Polynuclear Leukocytes** (Plate VI).—These cells are a little smaller than the large mononuclear leukocytes and represent the finely granular variety already mentioned. They are active phagocytes and as such capable of progressive locomotion. The nucleus in the younger cells is polymorphous, while the older cells are actually polynuclear, the number of lobes varying from two to six. In stained specimens the nucleus shows a coarsely reticular structure with nodal thickenings and is very markedly basophilic. The protoplasm, on the other hand, is very feebly oxyphilic.

Embedded in the protoplasm are numerous fine granules—the ε-granulation of Ehrlich—which are characterized by their affinity for neutral dyes. Hence the term polynuclear *neutrophilic* leukocytes.

These granules are ordinarily very abundant; but in disease they may diminish in number until very few are left, and in some cases they may, indeed, be absent. Ewing has called special attention to the decrease in the number of the granules in the acute leukocytoses. I have observed total absence of granules in a case of trichinosis at a time when marked eosinophilia existed. Kast mentions an instance of general carcinomatosis with a leukocytosis of 120,000, in which 1.68 per cent. of the cells contained no granules. Hirschfeld describes the same occurrence in connection with growths involving the bone-marrow, and others have noted it in myeloid leukemia, where toward the end, in chronic cases, it is a fairly common phenomenon.

Associated with the diminution in the number of the granules there are frequently also degenerative changes affecting the nuclei. These may be of the type of karyolysis with swelling and loss of chromatin, or of karyorrhexis with hyperchromatosis and fragmentation of the nucleus. The former is the more usual in the acute leukocytoses, while the latter is seen especially in leukemia. In cases of the myelocytic variety it is quite common to note complete fragmentation of the nucleus into from six to ten segments. This phenomenon was first observed by Ehrlich in a case of hemorrhagic smallpox, and is of common occurrence in fresh exudates. Cell degeneration associated with loss of chromatin and swelling, while it no doubt occurs to a greater degree in disease, may also be observed under normal conditions. In every dried and stained specimen a certain number of such cells will be found in which the nucleus appears as a much swollen and but faintly staining shadow, the *Kernschatten* of the Germans, sometimes surrounded by some of the granules, which appear scattered as though the cell had been burst asunder by force; at other times the Kernschatten alone remains and nothing is seen of the body of the cell.

I have stated that the loss of granules on the part of these cells may go on to a point where they are absent altogether. It may happen, however, that the granules are only apparently absent, and merely do not react as usual with ordinary dyes. A proper explanation of this peculiar behavior cannot be given, but every worker in blood is no doubt familiar with the phenomenon. Sometimes a change in the mode of fixation will cause the granulation to appear; at other times it may be demonstrated by the aid of some other dye.

Vacuolization of the polynuclear leukocytes is much less common than in the case of the mononuclear elements.

While the neutrophilic leukocytes, as a general rule, are large cells, unusually small specimens are seen in the blood of myelocytic leukemia. These dwarf forms must not be mistaken for the small cells which one may find in any specimen of blood where it is thick and where the process of drying has occurred slowly. In cells of this latter order the staining of the granules is also frequently deficient or they may not show at all.

The polynuclear neutrophilic leukocytes are derived from corresponding mononuclear forms—the neutrophilic myclocytes—which are normally found only in the bone marrow, and of which several generations can be distinguished. They result from the ontogenetically youngest generation directly and represent their adult form.

Arneth divides the polynuclear neutrophiles into five classes according to the number of the nuclear lobes. Under normal conditions the percentage numbers of the different varieties remain fairly constant for one and the same individual, but they vary somewhat in different people. The first class is represented by mononuclear cells and is subdivided into (*a*) mononuclear forms, corresponding to and identical with Ehrlich's *myelocytes* (see below); (*b*) forms with but slightly indented nuclei, the invagination not extending to a greater depth than the middle of the nucleus (the *metamyelocytes* or *proleukocytes*); (*c*) cells in which the invagination extends farther than in form (*b*), but in which no separation into isolated loops or lobes has as yet occurred—the true *polymorphonuclear* variety. The two first varieties are practically only seen under abnormal conditions, although an occasional metamyelocyte may at times be encountered in health. Cells of type (*c*) are normally present to the extent of 4 to 9 per cent. The second class comprises cells with two distinct nuclear segments, which may appear either as two loops or two lobes. They constitute from 21 to 47 per cent.; the number, as already stated, varies somewhat with the individual, but is quite constant for one and the same person. In this class the cells with two loops normally exceed those with one loop and one lobe, while true bilobes are rare. The third class shows three nuclear divisions and can be subdivided into four groups in accordance with the number of loops or lobes. Cells with two lobes and one loop numerically approximate those with two loops and one lobe, while cells with three loops or three lobes respectively are in the minority. Conjointly the groups of the third class represent 33 to 48 per cent. Their number thus about equals that of group two, but has a tendency to be somewhat larger. The fourth class is provided with four nuclear divisions with five subgroups and numbers 9 to 23 per cent. The fifth class finally comprises cells with five or more nuclear subdivisions and may be subdivided according to the same principle. Only 2 to 4 per cent. of the neutrophiles normally belong to this order. The various classes, just described. according to Arneth represent different stages in the development of the neutrophilic cells, the myelocytes on the one hand being the youngest, and the polynuclear leukocytes with many lobes the oldest. Arneth has shown that in disease marked deviations from these normal standards may occur, and that the qualitative changes may be most pronounced even though there be no quantitative changes in the total number of the leukocytes, and vice versa. He accordingly distinguishes between, *iso-, normo-, hyper-, and hypocytosis, and aniso-*,

normo-, hyper-, and hypocytosis, the terms *iso and aniso* having reference to a normal or abnormal nuclear picture, respectively. Arneth's results are very interesting and show conclusively that the absolute leukocyte count *per se* is relatively of little importance, and that a more detailed morphological study of the blood is necessary in order to derive all the information possible from the blood examination. I have myself insisted for years that of the two, the differential count is more important, and from my experience with Arneth's nuclear studies I am quite prepared to admit that his method will at times furnish information of value, even when the differential count shows but little abnormality.

When alterations in the nuclear picture do occur the change usually first affects the maturest forms, viz., group 5; then follow the others until finally the youngest forms largely remain. An anisohypocytosis, according to Arneth, represents the most serious condition so far as the leukocytic blood picture goes, as it indicates both an extensive destruction of leukocytes and a defective new formation. Less serious would be an anisonormocytosis, more favorable an anisohypercytosis, and most favorable an isohypercytosis.

Later investigators do not entirely support Arneth in this view nor in the conclusions which he has drawn from deviations from the normal numerical standards, in disease, but in practice a good deal of significance attaches to the observed facts.

While a tendency to oligomorphism of the nucleus is of common occurrence in pathological conditions the reverse is relatively rare. Brosamlen and Briggs have described this condition in connection with pernicious anemia, and the latter has pointed out that in a number of correspondingly severe cases of anemia, not of the idiopathic pernicious type, he found either a normal number, or a decrease in the number of lobes, a "shift to the left."

The polynuclear neutrophiles are the most common leukocytes of the blood and normally constitute from 60 to 70 per cent. of the total number. In young children they are relatively less numerous excepting during the first twenty-four hours of life, when they may number 73 per cent. But they rapidly diminish, so that values of from 20 to 40 per cent. may be regarded as normal during the first year. Low values continue practically to the twelfth year, though the numbers gradually rise. From the twelfth to the fourteenth year 60 per cent. may be regarded as an average; after that age the values given for the adult hold good.

An increase in the number of these cells is spoken of as a *neutrophilic polynucleosis.*

Leukocytic Inclusions.—Döhle has called attention to the fact that in scarlatina certain inclusions may be observed in some of the polynuclear neutrophilic cells, which in a measure are characteristic of the disease in question. Of their nature nothing is known. They

are round, oval, or crescentic little bodies, approximately of the size of a coccus, and may number from one to six per cell. They are said to appear even before the eruption and to persist for five or six days, after which they disappear. The number of cells which present these inclusions is variable, but, as a rule, quite large. During a recrudescence of the fever they do not reappear.

The inclusions can be demonstrated by staining with Manson's borax methylene blue solution (2 grams. of the dye dissolved in 100 c.c. of boiling 5 per cent. borax solution), with Giemsa's stain, pyronin, etc., the specimens being first fixed with alcohol. Subsequent investigators, while confirming the frequency with which the inclusions in question are seen in scarlatinal blood, have pointed out, however, that similar findings are common under other pathological conditions as well and that they can hence not be regarded as characteristic of scarlatina, or as directly connected with the disease in question. What their actual significance is remains to be seen. In the past I had looked upon the basophilic inclusions which one sees so frequently in pneumonia and severe pyogenic infections as possible remnants of phagocyted bacteria, but this also is a mere supposition. Others have found them in tuberculosis, measles, typhoid fever, etc.

4. **The Polynuclear Oxyphilic or Eosinophilic Leukocytes** (Plate VI).—In size and general appearance these cells resemble the polynuclear neutrophiles, and, like these, they are capable of progressive locomotion. The granules—the *α*-granulation of Ehrlich—however, are much larger and bleb-like, and possess a marked affinity for acid dyes, such as acid fuchsin and eosin. Hence the term *oxyphilic* or *eosinophilic* leukocytes. With neutral dyes or basic dyes they will not stain. The appearance of the individual granules varies somewhat in stained preparations. Some are round, others oval; some appear to stain throughout, others make the impression of little vesicles with a limiting membrane, which alone takes the dye, while the interior remains unstained. This bleb-like appearance of the granule is one of the most marked characteristics. Barker has shown that the granules contain iron. They are insoluble in ether and cannot be stained with osmic acid. They are, therefore, not composed of fat.

The protoplasm of the eosinophilic leukocytes is slightly basophilic, and usually almost altogether hidden from view, owing to the dense packing of the granules, thus differing markedly from what is observed in the neutrophile, where a background can always be discerned. The nucleus is mostly bilobed (spectacle nucleus), sometimes trilobed, and in stained specimens it is quite common to find the individual lobes unconnected by threads of chromatin; often the two lobes are situated at opposite poles. As a rule, the nucleus is less markedly basophilic than that of the neutrophilic variety. A nucleolus is not seen.

The same degenerative changes which have been described in connection with the polynuclear neutrophiles may also be observed in the eosinophiles, and here, as there, one can at times note a material diminution in the number of the granules. I have never observed their entire absence, however, and it is noteworthy that in those cases of chronic leukemia in which the neutrophilic granulation may disappear the eosinophilic variety remains.

While the common eosinophile is a large cell, unusually small forms are frequently seen in the blood of myelocytic leukemia. These should not be confounded with the small cells which may be seen in the thicker portions of almost any normal specimen, and which owe their small size to a gradual contraction during the process of drying.

Under normal conditions the percentage of eosinophiles varies between 1 and 4. An increase in their number is designated as *hypereosinophilia,* in contradistinction to *hypoeosinophilia,* which denotes a decrease.

While repeated attempts have been made to connect the eosinophilic leukocytes of the blood cytogenetically with the neutrophilic variety, there is no satisfactory evidence to support this view. On the contrary, there are strong reasons for believing that, analogous to the neutrophilic variety, the polynuclear eosinophiles are normally formed in the bone marrow, and here only, from mononuclear eosinophilic cells—the *eosinophilic myelocytes.*

5. **The Mast Cells** (**Polynuclear Basophilic Leukocytes**) (Plate VI).—The mast cells which are normally found in the blood are approximately of the same size as the polynuclear neutrophiles and eosinophiles. In myelocytic leukemia, however, in which they are especially numerous, the size is more variable; on the one hand, they may measure only 3.5μ in diameter, while on the other they may attain a dimension of 22μ. The nucleus is polymorphous; but the tendency to form individual lobes is far less marked than in the corresponding eosinophilic and neutrophilic elements. Quite commonly it is leaf-like and flat in appearance. Its affinity for basic dyes is quite feeble, so that it is often difficult in stained preparations to make out the boundary line between nucleus and protoplasm. It is almost always excentrically located and usually has a fairly uniform diameter of 4μ. In the smaller specimens the nucleus occupies almost the entire cell.

Embedded in the protoplasm lie granules of variable size—the γ-granulation of Ehrlich—some of which are fully as large as, or even larger, than the eosinophilic granules, while others are much finer. They are characterized by their affinity for basic dyes and the fact that with certain ones they stain metachromatically, viz., in a color which is different from that of the dye itself, which latter must be simple and not compound in order to bring out this point. Only a

limited number of dyes have metachromatic properties. The most notable ones are the violet basic dyes, hexamethyl violet, cresyl violet, thionin, neutral violet, and amethyst violet; further, the blue dyes, methylene azure, cresyl blue, and toluidin blue, and the red basic dyes, pyronin, acridin red, neutral red, and safranin. With the latter group the mast-cell granules are colored yellow, with most of the violet dyes red, and with cresyl violet R (extra) almost a pure brown. Methyl green does not stain the mast-cell granules unless it is contaminated with methyl violet, and for this reason the granules remain colorless in specimens stained with Ehrlich's triacid stain. In specimens fixed by heat and stained with aqueous alum hematoxylin solution the γ-granules are also not demonstrable. They have been dissolved; but there remains visible a well-defined spongioplasm, upon which the granules were deposited.

The mast-cell granules are *absolutely* basophilic, viz., they can only be stained with basic dyes, and retain the basic dye on subsequent differentiation in acid media. They are capable, moreover, of taking up the basic dye from its acidified solutions, as in the case of Ehrlich's dahlia-acetic acid mixture.

The granules of the common mast cells of normal blood are resistant to water, while in myelocytic leukemia cells are met with the granules of which dissolve with great readiness. Their chemical nature is still a matter of dispute, but there is a tendency to associate the mast cell with the formation of mucin. This presupposes the identity of the blood mast cell with the common mast cell of connective tissue. In the past this has been tacitly assumed, but Pappenheim more especially has called attention to the fact that the hematogenic mast cell differs from the histogenic form, and that the two probably represent different species. Pappenheim inclines to the view that the granulation of the hematogenous mast cell is not a true morphological granulation, but represents chemically altered lymphocytic spongioplasm or a transport substance which has been taken up and metabolized.

The number of mast cells varies between 0.2 and 1 per cent. Ewing states that he constantly failed to find mast cells in the better class of healthy subjects, while in hospital and dispensary cases with minor ailments they appeared to be more numerous. My own observations do not bear this out; in my experience they are invariably present in health irrespective of the general nutrition of the individual.

The origin of the mast cells of the blood has not been definitely ascertained. Ehrlich supposed that they originated from the connective-tissue cells as the result of hypernutrition, while Harris suggests that they may be derived from the large mononuclear leukocytes According to Pappenheim, the mast cell originates in the bone marrow from a granular mononuclear type which corre-

sponds to the eosinophilic and neutrophilic myelocytes, and thus indirectly from the large lymphocyte (Fig. 4).

Under Pathological Conditions the following Additional Types may be Encountered:

1. **The Lymphoidocytes** (myelolymphocytes, macrolymphocytes).—These cells represent the ancestral type from which all the different forms of leukocytes and red cells are derived through a process of differentiating reproduction. They are never found in the blood under normal conditions, but may be encountered in certain diseases (notably acute lymphatic leukemia and chronic myelocytic leukemia). They are normal inhabitants of the bone marrow.

Structurally the lymphoidocytes resemble the lymphocytes of the normal blood, but are usually greater in size. As in the case of the latter, the younger cells show only a very narrow rim of protoplasm, while in the older cells this is more extensively developed. Coincidentally, the basophilia of the protoplasm is more marked in the young cells than in the old ones. As in the case of the normal lymphocytes, moreover, the nucleus of the older cells shows a tendency to invagination. The essential difference between the lymphoidocyte and the lymphocyte of lymphadenoid origin is, however, not a question of size but of nuclear structure. This is best brought out on staining with a modern Romanowsky azure mixture. It will then be noted that whereas the nuclear chromatin of the lymphocyte proper appears more or less clumpy and pachychromatic, that of the lymphoidocyte is much more delicate, granular looking, and leptochromatic. The multiple basophilic nucleoli of the lymphoidocyte are usually also distinctly shown in this manner, while the lymphocyte proper commonly appears oligo- or anucleolar. It must be admitted, however, that slight variations in the duration of staining are often only too apt to eradicate these points of difference, and that in a concrete case considerable difficulty may be experienced in recognizing the medullary nature of what appears to be a lymphocyte proper. This is especially true of the small forms, the so-called microlymphoidocytes, which usually accompany the larger type, but may also control the entire blood picture.

The oldest forms of the macrolymphoidocytes, in which the protoplasm has become more extensively developed, closely resemble the large monocytes of the normal blood, and have in the past been mistaken for these. The presence of such cells in the bone marrow has given rise to the erroneous idea that the large monocyte is of medullary origin. Associated with the increased development of the protoplasm in these old forms there is frequently also an invagination of the nucleus of variable degree, exactly as we see it in the so-called transition forms of the large monocytes. Atypical karyomorphism (Rieder type) is only seen under pathological conditions.

Its appearance is the expression of an abnormal degree of cell regeneration in the absence of corresponding differentiation. (Plate III, *c*.) Such cells differ from the transition forms and the typically invaginated medullary leukoblasts (see below) not only in the marked pluripolar invagination of the nucleus, but above all in the marked degree of basophilia of the protoplasm. In other words, the nucleus has matured more rapidly than the protoplasm. They may contain azurophilic granules.

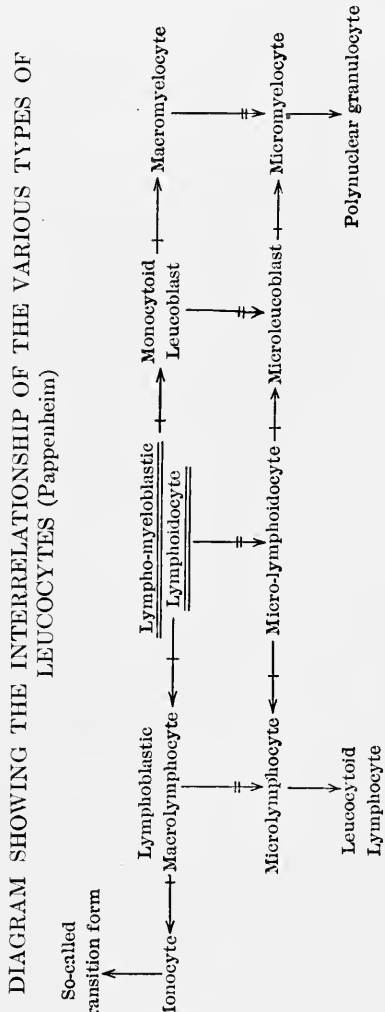


FIG. 4

2. **The Leukoblasts.**—These are cells which stand midway between the lymphoidocytes and myelocytes. They differ from the former in presenting a nucleus which bears all the earmarks of the myelocytic nucleus, viz., a striped or checkered arrangement of the chromatin with coincident loss of nucleoli, while the protoplasm itself is yet basophilic and free from specific granulations. Like the lymphoidocytes, however, they may present azurophilic granules. When the chromatin arrangement of the cells is as yet not typically myelocytic, *i. e.*, when the differentiation of the lymphoidocyte has not progressed sufficiently, they closely resemble the large monocytes of the normal blood, as do the adult and aged forms of the undifferentiated lymphoidocytes proper.

While certain writers in the past have not distinguished between lymphoidocytes and leukoblasts and have spoken of the two groups conjointly as *myeloblasts*, Pappenheim has pointed out that two groups of ancestral cells actually exist and that the term myeloblast can rightfully only be applied to the leukoblast.

3. **The Myelocytes.**—The myelocytes represent a further stage in the differentiation of the lymphoidocytes, by way of the leukoblasts, and differ from the latter in the presence of specific granulations. They may accordingly be defined as mononuclear granular leukocytes which normally are not found in the circulating blood, but may appear there under pathological conditions. During extra-uterine life they are the bone-marrow cells proper and the source of supply of the granulocytes. Three types are recognized, viz., those containing neutrophilic, eosinophilic, and basophilic granules. Corresponding to each type there are further certain subvarieties, which represent intermediary forms standing between the leukoblasts and the fully differentiated polymorphonuclear and polynuclear granulocytes of the circulating blood. Among the *neutrophilic myelocytes* we may thus distinguish between an *amblychromatic* and a *trachychromatic* form.

The *amblychromatic* (*macro-*) *myelocyte* is a large cell provided with a relatively large, centrally located, round nucleus which stains but feebly with basic dyes. This is surrounded by a comparatively narrow zone of basophilic protoplasm which contains very fine neutrophilic granules. As the cell matures the nucleus becomes more or less invaginated and ultimately polymorphous. The protoplasm at the same time becomes relatively more abundant. Pappenheim speaks of this type as the *promyelocyte*.

These cells do not become inhabitants of the normal blood themselves, but remain in the marrow as marrow cells proper, and here give rise to daughter cells of a smaller type, the so-called *trachychromatic* or *micromyelocytes*, which age into the polymorphonuclear neutrophilic leukocytes, and as such appear in the blood. This latter type is thus a

smaller variety, and essentially characterized by the greater staining power of the nucleus and the fact that its protoplasm is slightly oxyphilic, while the neutrophilic granulation at the same time is rather coarser than in the amblychromatic form. As this latter type matures the protoplasm becomes more abundant and the nucleus distinctly polymorphous; it then constitutes the common neutrophile of the circulating blood. Between these two extremes there are transition stages where the nucleus is still single, but already shows a marked tendency toward polymorphism. Such cells are termed *metamyelocytes* or *proleukocytes*, and do not occur in normal blood. They differ from the polymorphonuclear cells—so-called—in the fact that the nuclear segments are still united by broad bridges, instead of filaments of nuclear substance.

Neutrophilic myelocytes undergoing mitosis are sometimes seen in the circulating blood in myelocytic leukemia; on the whole, however, they are rare, and it is more common to meet with cells in which the division of the nucleus has already taken place (Plate VI).

Müller and Jolly have shown that the neutrophilic myelocytes of the circulating blood are capable of active locomotion.

The *eosinophilic myelocytes* which have nearly matured show a granulation which takes very nearly the pure eosin color on staining with eosin-methylene blue mixtures. The younger forms, however, present a purplish-violet color, and some granules may, indeed, be a pure blue (Plate VI). This appearance is owing to the fact that the young eosinophilic granule is physically cyanophilic and chemically oxyphilic, whereas the mature granule exhibits no physical chromatophilia and is merely chemically oxyphilic.

The protoplasm of the eosinophilic myelocytes is basophilic.

The size of the cells is quite variable; some are considerably larger than the corresponding polynuclear form, while others are much smaller. The most markedly cyanophilic cells are, generally speaking, the largest and may be viewed as the precursors of the smaller type.

The basophilic myelocytes, like the eosinophilic and neutrophilic varieties, may be of variable size and are provided with a large centrally located nucleus, which is often distinguished only with difficulty from the surrounding protoplasm.

The appearance of myelocytes in the peripheral circulation is spoken of as *myelocytosis.* The term *myelemia* may be conveniently used to express a large preponderance of these cells.

4. **The Lymphoblastic Macrolymphocytes.**—These cells represent the mother cells of the lymphocytes of normal blood, but are themselves only found here under pathological conditions. Their normal habitat is in the germinal centres of the lymph glands which are composed altogether of these cells. Structurally, they differ from

the small lymphocytes only in their greater size, which in turn is here dependent upon the greater size of the nucleus. Upon this basis they may also be distinguished from the leukocytoid (old) lymphocytes, in which the nucleus remains small while the protoplasm only increases in bulk and thus leads to the increased size of the cell. (Plate III, *a*.)

As the macrolymphocytes age they also develop an increase of their protoplasm, and as this occurs at the expense of the spongioplasm, leading to a diminished basophilia, such cells closely resemble the large monocytes, and it may indeed be impossible to differentiate them from these.

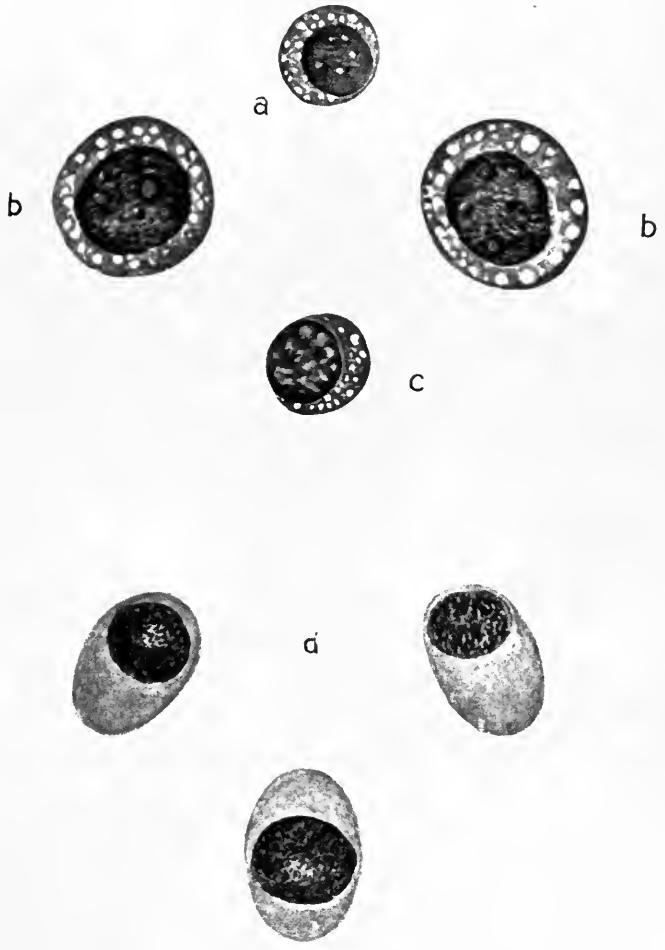
Like the small lymphocytes the ancestral type also is apt to carry azurophilic granules.

5. **Plasma Cells** (phlogocytes; pseudoplasma cells; irritation cells of Türck; stimulation cells).—These cells are essentially hematogenic lymphoid cells, whose spongioplasm has undergone an excessive development at the expense of the paraplasm (condensation, sclerosis). Morphologically their protoplasm is accordingly more markedly basophilic than in the corresponding normal lymphoid cells. According to Pappenheim, plasma cells may develop from any type of lymphoid cell, and he has pointed out that the origin of the individual cell is indicated by the structure of its nucleus, viz., this is leptochromatic, if of lymphoidocytic origin, pachychromatic and with a tendency to a radiary arrangement of the chromatin if of lymphadenoid source. Azurophilic granules are absent, but frequently the protoplasm seems literally honeycombed with vacuoles (Plate V). Pappenheim regards lymphocytes without a deeply staining (non-chromophilic) protoplasm, but with radiary nuclei as intermediary cells.

While these cells are essentially met with under pathological conditions, they are frequently seen in the normal blood of children, where they may represent 5 per cent. of all leukocytes. Pappenheim regards their occurrence, in disease, as indicating the existence of a chronic inflammatory process, either of the connective tissue, or of the hemopoietic apparatus (tuberculosis, Hodgkin's disease, myeloma, etc.). I have found them relatively numerous in inflammatory conditions of the abdominal viscera (peritonitis, appendicitis, typhoid fever), occasionally in measles, and most numerous in certain cases of myelocytic leukemia. A number of cases of leukemia have indeed been reported in which the predominating cell was a plasma cell. Pappenheim speaks of the occurrence of plasma cells with radiary nuclei in malaria.

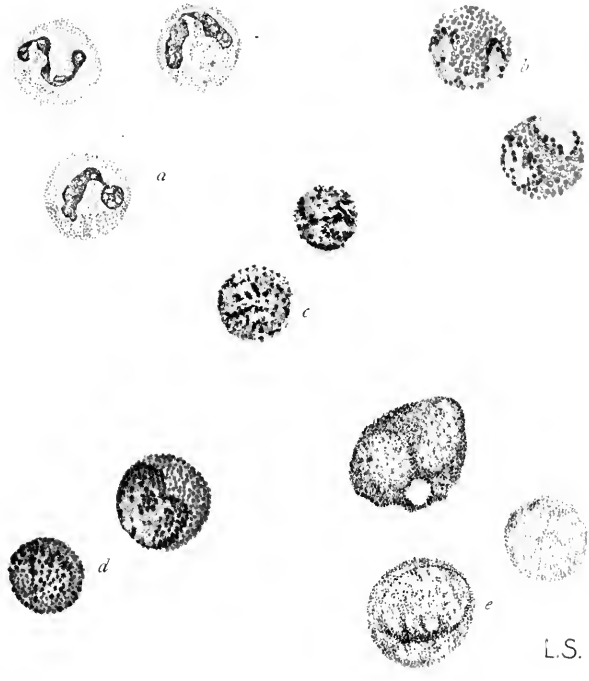
The term "irritation" or "stimulation" forms indicates that the cells are found in connection with infectious or toxic inflammatory "irritations."

PLATE V



a, microlymphoidocytic plasma cells; *b*, microlymphoidocytic form; *c*, lymphoblastic form; *d*, myelomatous plasma cells.

PLATE VI



Granulocytes.

a, polynuclear neutrophilic leukocytes; *b*, polynuclear eosinophilic leukocytes; *c*, mast cells; *d*, eosinophilic myelocytes; *e*, neutrophilic myelocytes (the smaller myelocytes represent the micro-, the larger ones the macro-type); *f*, the nucleus here has just undergone division; the clear space is a vacuole.

According to Pappenheim the occurrence of plasma cells in the blood is indicative of a chronic inflammatory process, either of the connective tissue or of the hemopoietic apparatus (tuberculosis, Hodgkin's disease, myeloma, etc.). I have found them relatively numerous in inflammatory conditions of the abdominal viscera (peritonitis, appendicitis, typhoid fever), occasionally in measles, and most numerous in some cases of myelocytic leukemia.

IODOPHILIA.

On staining blood-smears of normal individuals with iodin (see p. 74) the protoplasm of the leukocytes is colored a bright yellow, while the nucleus is somewhat refractory and takes on a lighter tint. Under certain pathological conditions this staining quality is modified; cells are then seen in which reddish-brown granules appear in the protoplasm, or it may occur that this presents a diffuse brownish color throughout. This intracellular reaction affects the polynuclear neutrophiles almost exclusively; the mononuclear elements *may*, however, also react, in which case one commonly sees large, pale brown granules arranged about the nucleus in a single row. In eosinophiles the reaction does not occur. The extent to which the leukocytes are involved is variable; in some cases a few cells only are affected, while in others one is scarcely able to find a normal cell in an entire preparation.

An extracellular reaction also occurs, but is of little clinical interest, as it is not infrequent even in health. The iodophilic material occurs in small, roundish or oval masses, which are possibly plaques, but which may, in part, be small bits of protoplasm derived from leukocytes.

As to the nature of the substance which reacts with the iodine in the manner indicated, there is no uniformity of opinion. Ehrlich regards it as glycogen, and assumes that this is present normally in every cell in the form of a colorless compound, from which the free glycogen is under certain conditions split off, and can then be demonstrated as such. Czerny, on the other hand, looks upon the iodophilic substance as an antecedent of amyloid, while Goldberger and Weiss view it as peptone. Kaminer has shown that normal bone-marrow does not contain iodophilic leukocytes, but that they may here be found when they are present also in the blood. He concludes that the reaction is a degenerative phenomenon and not an evidence of regeneration.

From the investigations of numerous observers it appears that septic conditions of all kinds may furnish a positive reaction, but that active suppuration may also occur without iodophilia. Locke's list of diseases of this order includes general septicemia, abscesses (excepting in the earliest stages), appendicitis accompanied by abscess

formation, general peritonitis, empyema, pneumonia, pyonephrosis, salpingitis with severe inflammation or abscess formation, tonsillitis, gonorrheal arthritis (in contradistinction to other forms), and acute intestinal obstruction where the bowel has become gangrenous. Locke concludes that no septic condition of any severity can exist without a positive reaction. In puerperal sepsis also it is said to be constant (Kaminer). In pneumonia with frank resolution it disappears in from twenty-four to forty-eight hours following crisis. In typhoid fever a positive reaction is not commonly obtained before the end of the second week, and it may, indeed, remain absent throughout the course of the disease. In the differential diagnosis between a serous and a purulent pleuritic effusion the absence of the reaction points to the former condition. Cerebral abscess may show the reaction, while in brain tumor it is absent (Gulland). In diphtheria it is only seen when there is much inflammation; it is never intense (Gulland).

In contradistinction to chlorosis, pseudoleukemia, and the common forms of secondary anemia of moderate intensity, iodophilic leukocytes are found only in the severer forms of anemia, such as pernicious anemia, leukemia (notably in acute cases), and the *severe* forms of secondary anemia.

LEUKOCYTOSIS

While the number of red corpuscles is normally fairly constant, that of the leukocytes is subject to variation. It is influenced by the age and sex of the individual, the process of digestion, menstruation, pregnancy, the bloodvessel from which the specimen is taken, etc. Generally speaking, the number varies between 3000 and 10,000, the exact number, *cæteris paribus*, depending upon the state of nutrition of the individual. In ill-nourished persons low values are the rule, while maximum numbers are generally associated with a state of exceptional vigor and good nutrition. These extreme figures, however, are uncommon, and, as a general rule, a count of 10,000 may be regarded as abnormal; 5000 to 6000 are the most frequent values which one finds if the examination is made with the individual in a fasting condition. During the process of digestion the figures are higher (see below).

An increase in the number of leukocytes is met with under the most diverse conditions, both in health and disease, and is designated as *leukocytosis*. But it would be better to restrict this term to indicate the number of leukocytes in a general way, and to speak of an increase as *hyperleukocytosis*, and of a decrease as *hypoleukocytosis*, or leukopenia.

It is important to remember that in disease an increase in the total number of leukocytes is never brought about by a simultaneous in-

crease of all the different forms which are normally present. When numerical results are obtained which would suggest such a conclusion, we may infer either that some technical error is responsible, or that we are dealing with a concentration of the morphological elements.

The largest increase in the number of leukocytes is met with in the leukemias. The count may here rise to 600,000 per c.mm., and even higher. This increase, in the myelocytic variety, is referable to an increased production of all types of granular leukocytes and to the simultaneous appearance in large numbers of the corresponding myelocytes. In lymphatic leukemia the absolute increase is, generally speaking, less extensive and brought about by an increased production of lymphocytes. Aside from these conditions, the most frequent form of hyperleukocytosis is of the ordinary neutrophilic type, next in order referable to lymphocytosis, then to hypereosinophilia and large mononucleosis, while the mast cells are never increased to such an extent as to cause an increase in the total number of leukocytes. The highest figures of course are met with in those forms of hyperleukocytosis which are caused by an increase of those cells which normally already are the most numerous, viz., the polynuclear neutrophiles and the lymphocytes. Exceptionally the values may approach those which are seen in leukemia, viz., 100,000 or more, but in the great majority of cases the increase is of a much lower order, usually ranging from 10,000 to 40,000; this latter figure, indeed, is already beyond the ordinary.

While in a general way the absolute values are proportionate to the intensity of the abnormal stimulus and the reactive power of the individual, this rule is hardly applicable to those pathological conditions in which the body responds with an increased production of eosinophiles, or of the large mononuclear leukocytes. The normal percentages of these types are so low that an ordinary increase in their production would hardly affect the total number. It is quite rare, in fact, to meet with leukocyte counts exceeding 10,000, in which the increase is of the eosinophilic or splenocytic type. On the other hand, conditions not infrequently arise in which the body does not respond with absolute hyperleukocytosis, though the stimulus be of a nature which ordinarily would cause such a result. This is notably seen in cases of pyogenic infection where the reactive power is defective but also in cases of unusually mild infections. If only a total count were made in such instances the underlying condition would be entirely overlooked.

No blood examination is accordingly complete in which the differential count has been neglected, and I would emphasize again and again that if only one count is for any reason to be made, it should invariably be the differential. It is a great surprise to me to see how little the importance of this dictum is as yet appreciated, and I would insist that the physician who nowadays does not resort to the differen-

tial count in every case that is clinically not absolutely clear, wilfully deprives himself of a diagnostic aid of the most important kind. There are few pathological conditions upon which it does not throw important light.

Neutrophilic Hyperleukocytosis.—As I have just indicated, neutrophilic hyperleukocytosis is especially apt to cause a marked increase in the total number of the leukocytes, and in a general way the relative percentage and the total number run a parallel course to each other. This type of hyperleukocytosis is most commonly seen in the pyogenic infections, viz., in infections with the streptococcus, staphylococcus, pneumococcus, meningococcus, catarrhal micrococcus, and the colon bacillus. The relative percentage depends primarily upon the intensity of the infection, while the absolute number may be viewed as an index of the reactive power of the individual. In infections of ordinary intensity with normal response absolute counts of from 15,000 to 30,000, with relative values of from 80 to 90 per cent., may be viewed as common values. Still higher values may be met with in especially severe cases. In my experience a relative count of 95 per cent. or over is of bad omen. The highest figure of which I have knowledge was 99 per cent. In those cases in which the individual apparently is overcome by the severity of the infection, from the very start, the absolute count is frequently little, if at all increased; there may, indeed, be a drop where before there had been a brisk hyperleukocytosis. In these cases particularly the differential count is apt to be very helpful, as it still reveals the existence of a severe infection, as evidenced by the relative increase of the neutrophiles.

When neutrophilic hyperleukocytosis is well marked it is common to meet with metamyelocytes in variable number; usually there are only a few, but at times their percentage may temporarily amount to anywhere from 5 to 10 and exceptionally even to more. The Arneth count at the same time reveals that the older cells (in the sense of Arneth, viz., those with three or more nuclei) have largely disappeared. This is especially significant in cases presenting a normal or nearly normal total count.

Associated with the increase of the neutrophilic cells in the pyogenic infections there is always a diminution or absence of the eosinophiles. This association I have designated as SEPTIC FACTOR. *If in a supposed infection of this order a normal or increased percentage of eosinophiles is observed the inference is warrantable, either that the infection is being successfully overcome or that a complicating factor is operative.* The other types of leukocytes are diminished, both absolutely and relatively. Apparent exceptions to this rule are rare (see section on Epidemic Meningitis).

A general idea of the various pathological conditions in which a neutrophilic hyperleukocytosis is a factor may be formed from a survey of the table on page 55.

Physiological Hyperleukocytosis.—A physiological increase of the leukocytes—physiological hyperleukocytosis—is notably observed at birth, during the process of digestion, in association with severe muscular exercise, following the use of cold baths, during the latter months of pregnancy and the puerperal state, etc., and requires a brief consideration.

Leukocytosis of the Newborn.—According to the experience of most observers, the number of leukocytes at birth varies between 10,000 and 23,000, of which over 70 per cent. are polynuclear neutrophiles. The number then falls and at the same time the lymphocytes increase. The curves of the two varieties cross between the sixth and the ninth day, and by the twelfth the lymphocytes are in excess. From the end of the first month to the fourteenth year there occurs a gradual increase of the neutrophiles and a decrease of the mononuclear elements. During the first year the total number of the leukocytes varies between 10,900 and 12,900; 9000 may be regarded as an average value from the first to the sixth year, and 7900 from then until the fifteenth year.

Digestive Leukocytosis.—The increase in the number of the leukocytes which is observed during the process of digestion affects both the polynuclear elements and the lymphocytes, though especially the latter. The eosinophiles are relatively at least diminished. The total increase rarely exceeds 3500 in normal adults, while in young children it may be much more marked. Schiff cites an instance in which 19,500 leukocytes were counted one hour after birth, 27,625 after the first meal, and 36,000 after the fourth meal. It is especially pronounced after a preliminary period of fasting and following a meal rich in proteins. The maximum increase is usually observed between the third and fourth hours.

In cases in which a hyperleukocytosis exists from other causes, as in pregnancy, in inflammatory diseases, etc., digestive hyperleukocytosis does not occur. Lobenstine, in analyzing 20 cases of pregnancy in this direction, found digestive leukocytosis in 13, no change in 1, and an actual decrease in 6. Apparently, however, he only made his examinations following the ordinary mid-day meal. In a few isolated instances it has also been found absent in apparently normal individuals without assignable cause. Under pathological conditions its absence is not uncommon, even though hyperleukocytosis referable to other factors may not exist. This is notably the case in carcinoma of the stomach (which see), and it was once thought that the absence of digestive hyperleukocytosis in doubtful cases could be interpreted as evidence in favor of its existence. In anemic individuals, from whatever cause, especially large amounts of proteins are sometimes necessary to elicit a digestive increase of the leukocytes, and in some cases a subnormal number even may be encountered.

To study digestive hyperleukocytosis, it is well to proceed as follows:

(*a*) The first blood count should be made after the patient has fasted for about seventeen hours.

(*b*) After this period he receives a test meal consisting of from 200 to 1000 c.c. of milk and one or two eggs, the amount varying with the condition of the patient.

(*c*) Further blood counts are made one, two, three, and four hours later.

(*d*) The existence of a digestive hyperleukocytosis should only be regarded as proved if an increase of at least 1500 cells occurs, providing that maximal amounts of food have been taken. If smaller amounts have been given, an increase of 1000 cells is sufficient to establish its existence, provided that the same result is observed on repeated examination.

On a diet of sugar and carbohydrates exclusively the digestive leukocytosis is essentially of the lymphocytic type.

As in digestive leukocytosis, the *hyperleukocytosis of pregnancy and the puerperal state* is brought about by an increase both of the polynuclear neutrophiles and the lymphocytes, while the eosinophiles remain passive. (See section on the Puerperal State.)

Leukocytosis following Baths, Muscular Exercise, etc.—The increase of the leukocytes following cold baths may, according to Thayer, amount to nearly 300 per cent. In 20 cases of typhoid fever he found 7724 leukocytes on an average before and 13,170 after the usual Brand bath. In his own person, while in health, the leukocytes on one occasion numbered 3250 before the bath, while twenty minutes later they had increased to 12,500. Such an increase is, however, only observed after a bath of moderate duration, while a prolonged cold bath diminishes the number. Hot baths have exactly the opposite effect, viz., those of short duration produce a decrease, those of long duration, an increase. Differential counts have, unfortunately, not been made.

Active muscular exercise produces a temporary hyperleukocytosis, and Grawitz has recently shown that this *myogenic form* is not referable to altered distribution of the cells, as was formerly supposed, but to actual increased production, and is of the neutrophilic type. The increase in Grawitz's experiments amounted to a plus of from 5700 to 10,900 cells. He is inclined to ascribe the hyperleukocytosis occurring during labor to the same cause.

Neutrophilic Leukopenia.—Just as hyperleukocytosis in the majority of cases is due to an increase of the polynuclear neutrophiles, so is leukopenia usually referable to a diminution of these elements in the blood. The extent to which this may go is variable. In the majority of the diseases in which leukopenia plays a role it is rare to meet with a decrease below a thousand, but occasionally this is seen. Vickery mentions a case of splenic anemia with a count of 650; Strauss and Rohnstein cite two cases of pernicious anemia with counts of 400 and

328 cells respectively. One of the lowest counts on record is given by Selling, in a case of purpura hæmorrhagica due to benzol poisoning, viz., 140. On rare occasions still more remarkable instances of leukopenia have been encountered. Ehrlich thus cites the case of a well-built young man in whom brief epileptiform seizures occurred, and in one of which the patient died. The postmortem examination was entirely negative. During the three days preceding death two examinations of the blood were made. On the first not a single leukocyte could be demonstrated in ten blood films, and on the second day but one was found in the same number of specimens.

Of *drugs*, atropin, camphoric acid, tannic acid, picrotoxin, agaricin menthol, sulphonal, and several other antihydrotics cause a marked decrease of the leukocytes.

The more important pathological conditions in which leukopenia has been observed are given in the table on page 55.

Eosinophilic Hyperleukocytosis.—As I have indicated before, it is exceptional to meet with a material increase in the total count which has been brought about by an increased production of eosinophiles. Nevertheless it occurs, but it is observed almost exclusively in the severer forms of trichinous infection. Brown mentions a case in which the total count was 35,000. Much more common is a relative increase only, which may, however, be very considerable. One of the highest relative counts of which I have knowledge has been reported by Kerr—viz., 86.6 per cent.—also in a case of trichinosis. In the majority of cases of hypereosinophilia the increase is much more moderate, ranging between 15 and 30 per cent.

Hypereosinophilia is of special clinical interest from the fact that it is observed in relatively few diseases (see table on page 55) which can be readily distinguished from each other. It is hence of high diagnostic importance.

Hypoeosinophilia.—Hypoeosinophilia has not received the attention which it deserves. As a result of my own studies in this direction, which now extend over many years, I would formulate the general rule that a diminution in the number of the eosinophiles will be observed at some period in the course of anyone of the various bacterial infections, no matter whether they are associated with a general polynuclear neutrophilic hyperleukocytosis or not. The extent to which this may go is variable; in the milder infections the values are but little, if at all, below the minimal normal, but in the severer and more protracted cases not a single eosinophile may be met with in a count of a thousand cells or more. Whether or not cases occur in which they are wholly absent from the circulating blood I am not prepared to say. I have pointed out before that I designate a neutrophilic increase, when associated with an eosinophilic decrease, as the *septic factor*, and regard this as one of the most valuable symptoms of the existence of a pyogenic infection. I would emphasize that in

active appendicitis, for example, the septic factor is of constant occurrence and will serve to differentiate the condition from other—non-pyogenic—abdominal diseases. On the other hand, I always suspect some complicating factor, such as a breaking-down malignant growth or a tubercular lesion when I meet with a neutrophilic increase in association with normal or slightly increased eosinophile values. During convalescence from pyogenic infections normal eosinophile values may be observed, even though the neutrophiles have not yet returned to the normal. The number of eosinophiles is hence of a certain prognostic significance; *their return in conditions in which they have previously been absent or diminished is a good omen.*

Lymphocytosis.—Lymphocytosis stands next to neutrophilic polynucleosis in the order of frequency of occurrence, and often is of such grade as to cause an absolute hyperleukocytosis. The highest counts of this order are met with in the lymphatic types of leukemia, where the total count may rise to 200,000 or even higher. With this exception, the values are usually much more moderate (10,000 to 20,000), and even more frequently we meet with only a relative increase. The relative number may range from normal to 90 per cent. or more. Figures above 70 per cent. are, however, unusual excepting in lymphatic leukemia. Practically important is the fact that a lymphocytic increase is not met with in infections with the pyogenic organisms, viz., the streptococcus, staphylococcus, pneumococcus, meningococcus, and colon bacillus.[1] When lymphocytosis is noted in a febrile affection, one should always suspect influenza, tuberculosis, typhoid fever, or whooping-cough. Of the non-infectious diseases, those affecting the ductless glands are especially apt to be associated with lymphocytosis (Basedow's disease, disease of the hypophysis and adrenals, status thymolymphaticus). (The various pathological conditions which are associated with lymphocytosis are enumerated in the table on page 55.)

In almost all cases in which lymphocytosis occurs it will be noted that this is of the small mononuclear type. Macrolymphocytosis is seen as a predominating condition only in acute lymphatic leukemia.

An experimental lymphocytosis has been observed following the injection of tuberculin and of extract of carcinomatous tissue (Grawitz). Waldstein claims to have produced a marked increase of the lymphocytes by hypodermic injections of pilocarpin, but, according to Ewing, this increase is only relative and brought about by a diminution of the polynuclear cells. Wilkinson speaks of a lymphocytosis following the injection of quinine hydrochlorate, and Perry has noted the same after the administration of thyroid extract.

[1] From this general rule I would except occasional cases of streptococcus infection for which I cannot account at the present time. Possibly special strains may be concerned, but our knowledge of this subject is as yet too meagre to warrant its further discussion at this place.

TABLE SHOWING THE MOST IMPORTANT DEVIATIONS FROM THE NORMAL LEUKOCYTIC FORMULA IN SOME OF THE MORE COMMON DISEASES.

Neutrophilic hyperleukocytosis.	Neutrophilic leukopenia.	Lymphocytosis.	Lymphopenia.	Hypereosinophilia.	Hypoeosinophilia.	Splenocytosis	Hyperbasophilia.	Myelocytosis.
All pure infections with streptococci, staphylococci, pneumococci, meningococci, catarrhal micrococci, and colon bacilli; hence the common wound infections, pneumonia, erysipelas, meningitis, peritonitis, appendicitis, tonsillitis, salpingitis, puerperal endometritis, diphtheria, acute rheumatism, empyema. Various abscesses. Eclampsia; following hemorrhages; scarlatina, etc.	Typhoid fever, paratyphoid fever, measles, uncomplicated tuberculosis, influenza, pernicious anemia, splenic anemia, etc.	Acute and chronic lymphatic leukemia, whooping-cough, rickets, congenital syphilis, measles, typhoid fever, tuberculosis, influenza, paresis. Diseases involving the ductless glands (Basedow, diseases of the hypophysis, adrenals, status thymolymphaticus).	The various diseases which are associated with an increase of the polynuclear neutrophiles.	Myelocytic leukemia, bronchial asthma, scarlatina, various skin diseases, various intestinal parasitic diseases (hookworm disease, trichinosis, etc.), gonorrhea, certain cases of tuberculosis, during convalescence from the pyogenic diseases mentioned in column I.	This is noted in all those conditions which have been mentioned in column I.	von Jaksch's anemia, rickets, syphilis, typhoid fever, smallpox, mycosis fungoides, chronic malaria, kala-azar.	Myelocytic leukemia, Asiatic cholera.	Myelocytic leukemia. The various pyogenic infections mentioned in column I. Pernicious anemia, severe secondary anemia.

Under physiological conditions, lymphocytosis is essentially noted in early childhood and in connection with the neutrophilic increase which occurs during the process of digestion.

Lymphopenia.—Lymphopenia is notably observed in the acute infections which are associated with an increase of the polynuclear neutrophiles, and is almost always relative. The condition *per se* has received but little attention, and is relatively unimportant from the clinical standpoint.

Splenocytosis.—Splenocytosis has received comparatively little attention. Clinical interest centres in its occurrence in chronic malaria, kala-azar, in certain cases of v. Jaksch's anemia, and in the later stages of typhoid fever. The total number of the leukocytes is only exceptionally affected (v. Jaksch's anemia), but the relative values may be quite high; 20 to 30 per cent. are common values. Recently I counted 75 per cent. in a late case of ambulatory typhoid (see also table on page 55).

Mast-cell Hyperleukocytosis (Hyperbasophilia).—This is a constant symptom of myelocytic leukemia, but in itself would be insufficient to cause an increase in the total number of the leukocytes. In the disease in question the percentage may rise to 15 or even higher. In other pathological conditions it is rare to meet with values higher than 2 per cent.; the cells are usually absent when the eosinophiles are low.

Myelocytosis.—Under strictly normal conditions, myelocytes are not found in the circulating blood. Small numbers of neutrophilic cells—up to 5 per cent.—are common whenever there is a brisk polynuclear neutrophilic increase. This is seen especially frequently in children where somewhat higher percentages even may be temporarily met with (up to 20 per cent.). The same may be observed in severe types of anemia, though the number is here usually small. The cells in question which may be found under such conditions are for the most part metamyelocytes of the small trachychromatic type. Amblychromatic neutrophilic myelocytes and *leukoblasts* are very rarely, if ever, seen excepting in myelocytic leukemia, which is the one condition above all in which myelocytes of all kinds appear in the blood. The enormous increase in the total number of the leukocytes which is there seen is, indeed, to a large extent due to these cells (myelemia). Their number is often most remarkable and a count of 50,000 to 100,000 per c.mm. by no means exceptional. The average percentage noted by Cabot in eighteen cases was 37.7, corresponding to a total of 162,000 leukocytes.

Eosinophilic myelocytes are rarely seen excepting in myelocytic leukemia, where their number usually ranges between 3 and 6 per cent. Isolated cells of this order have been met with in isolated cases of other diseases, but are of no special interest. Exceptionally small cells which have been noted by several observers may not have been myelocytes at all, but fragments of adult cells which have become

separated; I have seen this myself in a few instances (see also table on page 55 and special chapter on Myelocytic Leukemia).

The Leukocytosis of Childhood.—It has been pointed out before that in the blood of normal adults the small mononuclears range between 20 and 30 per cent., the large monocytes between 1 and 6, the neutrophiles between 60 and 70, the eosinophiles between 1 and 4 and the basophiles between 0.5 and 1 per cent. Any deviation from these numbers may be viewed as abnormal. In childhood, on the other hand, the normal percentages are different, there being a tendency toward higher values of the lymphocytes and a correspondingly lower value of the neutrophiles. This is very important to bear in mind in interpreting the differential counts in children younger than eight years, and for convenience of reference I here append the table of normal values given by Peabody, Draper and Dochez.

Differential Blood Counts in Normal Individuals.

Age.		Small mononuclears.	Large mononuclears.	Polymorphonuclears.	Eosinophiles.	Basophiles.
6 to 12 months	Maximum	58.5	12.2	35.9	4.5	0.8
	Minimum	50.5	7.3	24.6	0.0	0.1
	Average	55.9	9.6	30.4	2.6	0.4
1 to 2 years	Maximum	58.8	11.7	39.7	6.0	0.5
	Minimum	45.3	6.7	27.5	1.6	0.0
	Average	51.2	8.5	36.3	3.2	0.2
2 to 3 years	Maximum	55.0	11.3	44.3	6.0	1.2
	Minimum	43.5	5.0	33.2	0.5	0.0
	Average	49.0	8.2	38.7	3.1	0.4
3 to 4 years	Maximum	47.6	16.2	54.1	4.2	0.9
	Minimum	32.2	5.0	36.2	1.5	0.0
	Average	39.1	11.2	44.7	2.8	0.5
4 to 5 years	Maximum	49.5	6.7	51.7	4.0	0.6
	Minimum	38.4	3.4	42.2	1.6	0.3
	Average	42.1	6.0	48.5	2.6	0.3
5 to 6 years	Maximum	36.7	16.0	61.8	4.7	1.0
	Minimum	21.2	6.5	52.6	0.7	0.3
	Average	29.9	10.0	56.5	2.5	0.6
6 to 7 years	Maximum	34.1	15.7	61.3	4.7	0.6
	Minimum	24.5	8.1	52.3	0.1	0.0
	Average	30.4	10.8	56.0	2.2	0.2
7 to 8 years	Maximum	39.1	15.2	72.0	3.5	0.2
	Minimum	21.1	6.7	45.2	0.0	0.0
	Average	32.5	11.6	54.4	0.1	0.06

In commenting upon these figures I would merely say that in my estimation eosinophile values lower than 1 per cent. are always abnormal and that I cannot recognize the minimum figures given, which are lower than 1, as normal. Possibly the writers did not count a sufficient number of cells to secure a fair average.

If then in a child the question of a septic infection arises, one should

always bear the normal number of neutrophiles in mind, corresponding to a given age, and realize that an increase of 10 and 25 per cent. in a child may not bring the number of neutrophiles even up to the normal adult values, while in reality it would correspond to an adult polynucleosis of 80 to 85 per cent. Low eosinophile values should be viewed with particular suspicion.

THE PLAQUES

In addition to the leukocytes and red corpuscles, large numbers of small, roundish elements are encountered in the blood, which measure about 3μ in diameter and are free from coloring matter (Plate II, *l*). They are frequently seen collected into groups resembling bunches of grapes. These are the blood plates or plaques of Bizzozero.

Origin.—Lilienfeld, Hauser, Howell, and others regard the plaques as disintegration products of leukocytes, while others look upon them as precipitated globulins derived in part from the morphological elements of the blood and in part originating directly in the plasma. More generally accepted is the view expressed by Engel, Bremer, Maximow, Pappenheim, and others, according to which the plaques are derived from the red cells by extrusion. They are supposedly present in the interior of the cells as so-called nucleoids, and represent the remains of the original nucleus, which has lost its individuality as the result of chromatolysis. As a matter of fact, it is possible by suitable staining to demonstrate structures of this order not only within the red cells, but also their extrusion from the cells, so that the erythroglobular origin of some of these formations at least can scarcely be doubted. Jost, moreover, has shown that in the blood of sheep and calf embryos they appear at a time when leukocytes are not as yet demonstrable. But, on the other hand, there is a possibility that what we generally designate as plaques does not represent a unity, and that some of the elements which resemble the true blood platelets may have a different origin. To a certain extent such ill-defined little bodies are without doubt derived from leukocytes by a process of plasmorrhexis—*i. e.*, by the liberation of small bits of protoplasm. This may be observed under the microscope directly.

Deetjen claims that the true plaques are capable of executing ameboid movements, and that they are nucleated; he concludes that the bodies in question do not represent artefacts or products of degeneration, but are true cellular elements.

Wright finally believes to have proven that they are detached fragments of the cytoplasm of the megakaryocytes of the bone-marrow.

Variations in Number.—According to Osler, the number of plaques varies normally between 200,000 and 500,000 per cubic millimeter. Brodie and Russell claim that this number is too small, and that with

their improved method of counting, an average of 635,300 is obtained. The normal ratio between the plaques and the red corpuscles would thus be 1 to 7.8, taking 5,000,000 as the average normal for the red cells. More recently Helber found variations between 192,000 and 264,000.

Under pathological conditions the plaques may be increased or diminished. In pernicious anemia their number is very low; Van Embden found 64,000 and 32,000 in two cases. At times they are apparently absent, but in some cases increased numbers have been observed.

According to Pappenheim the plaques are diminished in pernicious anemia owing to over-rapid maturation of the red cells. As a result the nuclei of the erythroblasts either do not become pyknotic and undergo chemical chromatolysis with consequent formation of oxyphilic, viz., azurophilic nucleoids, but are destroyed already at an early stage by karyorrhexis; or, if they do become pyknotic, they are expelled from the cells plasmolytically in the anisotonic (anemic blood serum). A nucleoid thus does not remain which could later escape as a plaque.

In leukemia the plaques are often greatly increased. A large increase is at times observed in posthemorrhagic anemia and in chlorosis, but the results are not constant. In the secondary anemias referable to carcinoma, sepsis, tuberculosis, etc., the findings are variable; sometimes an increase is observed, at others a decrease, and then again normal values; the results, moreover, are inconstant in one and the same case. In the acute infectious diseases their number is the smaller the more severe the course of the disease. In pneumonia they are often diminished during the fever, but increased after the crisis. Similar results have been obtained in typhoid fever, while in erysipelas they are found increased from the start. Enormous numbers of plaques may be seen in the course of trichinous infections. Schleip looks upon their appearance in large numbers as evidence of approaching convalescence. In one of my own cases, however, they seemed to be most numerous at a time when the clinical symptoms were most active.

In hemophilia the number of blood platelets is normal, while in the purpuras it is diminished—an important factor in the differentiation between the two conditions.

THE DUST PARTICLES OR HEMOKONIA OF MÜLLER

These may be seen in any fresh specimen of blood mounted in the usual manner. They are small, generally round, sometimes dumbbell-shaped, colorless, highly refractive granules, which manifest very active molecular movements. They occur in the plasma of the blood and are apparently not connected with the process of coagulation. Müller found them abnormally numerous in a case of Addison's

disease, while they were diminished during starvation and in various cachectic conditions. Stokes and Wegefarth regard these granules as identical with the neutrophilic and eosinophilic granules of the leukocytes. They suppose that the bactericidal power of the leukocytes and of the serum of man and many animals may be due to their presence. I have quite constantly found the hemokonia increased at the height of digestion.

GENERAL EXAMINATION OF THE BLOOD

GENERAL MICROSCOPIC TECHNIQUE

Slides and Cover-glasses.—To obtain the best results, it is essential to have glassware of the best quality. The cover-glasses should not measure more than 0.08 to 0.1 mm. in thickness, and must be cleansed with care. The same holds good for the slides. With many slides it will be found that one side is more or less convex; if by chance this side is placed on the stage of the microscope, the specimen will be somewhat difficult to manipulate under the oil immersion, while with the other side down it will be much easier to work.

Both covers and slides are best cleansed by placing them in concentrated sulphuric acid or in glacial acetic acid for several hours. They are then thoroughly washed in running water and distilled water and placed in alcohol and finally in ether, where they remain for several hours. During this process care must be had that they are well separated from each other. Subsequently they are kept in jars with absolute alcohol, and are dried just before use, or they may be dried at once with fine linen or Japanese lens paper and stored in dust-proof receptacles. When once cleansed the cover-glasses should be handled only with forceps.

The Blood Mount.—We distinguish between wet mounts and dry mounts. Wet specimens can only be utilized successfully if the patient is near at hand to the laboratory, as in office work and in the hospital; where several hours must elapse before the preparation can be examined, it will be best to resort to the dry specimen. Wet preparations, however, are very convenient and yield a large amount of information without delay, and a rapid survey will indicate whether or not it will be necessary or advisable to resort to a more detailed examination. The grade of anemia; the degree, character, and extent of leukocytosis; the presence of malarial organisms, can all be told from the wet preparation. With the dry and stained specimen, on the other hand, all these points are brought out more distinctly, and other information is further afforded which cannot be obtained from the wet specimen alone.

To prepare a blood specimen, the tip of a finger, or in children especially the lobe of the ear or the big toe, is first cleansed with

alcohol and then punctured with a suitable instrument, such as a fine lancet or a Hagedorn needle. The puncture should be sufficiently deep that the blood will flow from the wound without undue pressure.

Preparation of Wet Specimens.—To prepare a wet specimen, a clean cover-glass is taken up with a pair of forceps with flat blades and a light spring, touched to the drop without coming in contact with the skin, and immediately transferred to a clean slide. If suitable glassware is used that is perfectly clean, the drop will immediately spread out between cover-glass and slide, and on *examining with a low power, which should always precede examination with a high power,* it will be noted that in the central portion of the specimen especially the red cells will be well separated from one another and will not have run into rouleaux. This will only occur if the glassware is imperfect, if it is not perfectly clean, or if the drop has been too large. To gauge the proper size of the drop requires a little practice. Along the margin of the specimen, where a certain amount of evaporation is going on, it is usual to find rouleaux and crenated red corpuscles, even though the remainder of the specimen be perfect, and in the course of time postmortem changes will also become noticeable throughout the preparation. If the specimen is ringed with a little vaselin, however, a satisfactory examination is still possible after a number of hours, and even without being ringed such preparations can be kept for at least one hour.

Preparation of Dry Specimens.—To prepare dry specimens, which are subsequently to be stained, the blood is spread between cover-glasses or on slides.

Personally, I have abandoned the use of cover-glasses for this purpose, and much prefer slides for routine work. A little practice only is required to obtain satisfactory results, and it is possible to control the quality of the individual smears with a degree of precision which is but rarely attained even by the most experienced workers with cover-glasses. The spreads, moreover, are much larger, so that there will always be a sufficient number of leukocytes available even under normal conditions to permit a count of at least a thousand cells. At the same time it is possible to spread portions of the drop so thin that the individual cells are well separated, the one from the other, while other portions can be made a little thicker. The slides are best cleansed in the same thorough manner as in the case of the cover-glasses, although one can get along with glassware that has been well washed with soap and water. A fair-sized drop of blood is mounted near the end of one slide and spread with an even sweep with the edge of a second slide; this should be done with a *light* hand, and holding the first slide in the left hand between the thumb and the second and third fingers. The second slide should also be held in this manner, but at an angle of 45 degrees to the first, as shown in the accompanying illustration (Fig. 5). Before commenc-

ing the sweeping movement, I let the blood spread along the edge of the second slide by capillary attraction; then I move across, *gradually raising the second slide to a vertical position, so as to spill the drop, as it were, while spreading. Pressure must be carefully avoided.*

Fig. 5.—The preparation of blood smears on slides.

If covers are to be used, one cover-glass is locked in a pair of forceps such as those devised by Ehrlich and pictured in the accompanying illustration (Fig. 6). A second cover is taken up with a pair of forceps without a lock, but with flat blades and a light spring; this is held to the drop of blood just as it emerges from the puncture, and is then immediately laid upon the first cover. If the glasses are of satisfactory quality and chemically clean, the blood will at once spread in a capillary layer; the top cover is then drawn from the lower

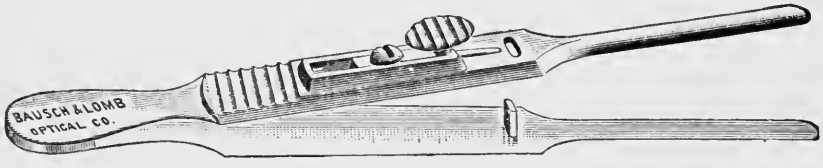


Fig. 6.—Ehrlich's cover-glass forceps.

cover by grasping the edge firmly with the fingers and making even traction in a plane parallel to the other. Here also a certain amount of experience is necessary in gauging the size of the drop in reference to the size of the covers. In no case should it be so large that the top cover *floats* upon the blood. If the drop is rather small, the two covers

should overlap only to such an extent as to furnish a space which is just filled by the blood. If the drop is larger, they should overlap over a larger surface.

After being allowed to dry in the air, the blood films may be placed on top of each other, wrapped in paper, and can then be stained when at leisure. If several days must elapse before the examination, it is well to place them, wrapped in filter paper, in closed jars. Should it be desired to preserve the specimens for a long time—*i. e.*, for months or years—it is best to coat the films with a thin layer of paraffin, which later is dissolved by immersion in toluol. In this manner especially valuable and rare specimens may be kept almost indefinitely. Unless this precaution is taken, the staining qualities of all the morphological elements of the blood will undergo changes which will render the specimens unfit for color analysis.

Fixation.—With the majority of the blood stains which are now in use special fixation is not required, as the stains in question are strongly alcoholic, the alcohol fixing during the process of staining. With non-alcoholic stains, however, the films must previously be fixed. In the days when Ehrlich's triacid stain was in common use this was usually done by heating on a copper plate (10 cm. by 40 cm. by 3 to 5 mm.), the specimens being placed at a point where the temperature ranged between 100° and 126° C. (ascertained by a series of drops of water, toluol—boiling point, 110° to 112° C.—or xylol—137° to 140° C.—etc., and noting the line at which ebullition occurs). If the distance of the plate from the flame and the size of the flame, etc., are constant, the apparatus requires practically no attention and serves its purpose very well. An exposure for a few minutes to a half-hour, or even longer, was demanded. Nowadays this complicated technique is fortunately no longer necessary. If aqueous solutions are to be used for staining purposes fixation for five to ten minutes in strong alcohol will be found to answer all purposes, after which the specimens are rinsed in water and are then ready for staining.

Formalin also is useful as a fixing agent, and may be used in connection with many of the common blood stains. A 1 per cent. solution of the liquid commercial formalin is employed, in approximately 50 per cent. alcohol. Fixation is completed in one minute, and for practical purposes it is merely necessary to cover the blood films with a few drops of the solution, which is then drained off and replaced with the staining reagent directly. The method is not to be recommended, however, for routine purposes, as it interferes with various stains and often changes the normal chromatophilia. The same may be said of the use of concentrated solutions of bichloride of mercury, which also is useful for some purposes, but not for routine work.

The Anilin Dyes and Principles of Staining.—The anilin dyes with which we have to deal in the clinical laboratory are all

derivatives of hydrocarbons and all contain the benzol ring. Their staining properties are dependent upon the presence in the individual compounds of two distinct atomic complexes which are spoken of as *chromophoric* and *auxochromic* groups respectively. The presence of the chromophoric group imparts chromogenic properties to the substance, the dye itself resulting on the further introduction of an auxochromic group. The auxochromic groups are salt-forming radicles and render the dye either basic or acid. Two markedly auxochromic radicles are known, viz., the strongly basic amino group $—NH_2$ and the feebly acid hydroxyl group —OH. Still other salt-forming radicles may enter into the composition of the dye, but it is noteworthy that these have but feebly developed auxochromic properties. Radicles of this order are notably the carboxyl group —COOH, the sulphoxyl group $—SO_2OH$, the nitro group $—NO_2$, and the nitroso group —NO (which two latter may also occur as chromophoric radicles). As the chromophoric radicle itself may have acid or basic tendencies, it is manifest that the ultimate reaction of the individual compound will depend upon the interrelation of the sum of its acid and basic radicles. Markedly acid dyes will result if both the chromophoric group and the salt-forming radicles are acid, while strongly basic dyes will be the outcome if both have basic tendencies. Between these two extremes various possibilities exist, the ultimate reaction depending upon the character of the chromophore, the presence of acid or basic salt-forming radicles, the simultaneous presence of both, their number, etc. We may accordingly divide the various dyes into the following classes:

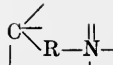
1. Basic amino dyes.
2. Acid nitroso dyes.
3. Acid sulpho- and nitro dyes, viz., amino- or oxysulphonic acids, aminoöxysulphonic acids, nitrophenols, nitroamins, nitroaminosulpho acids, nitroöxysulpho acids, nitroaminoöxysulpho acids.
4. Acid oxy- and oxycarbonic dyes.
5. Aminoöxy-, aminocarbonic, and aminoöxycarbonic dyes.
6. Aminosulphocarbonic-, oxysulphocarbonic-, aminoöxysulphocarbonic-, aminonitrocarbonic-, oxynitrocarbonic, aminoöxynitrocarbonic-, and aminoöxysulphonitrocarbonic dyes.

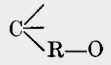
Of chromophoric groups, some twenty are known, and it is customary to classify the anilin dyes on the basis of these underlying radicles. We thus find:

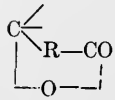
The $—NO_2$ group in the nitro dyes (picric acid, Martius yellow, naphthol-yellow S, aurantia).

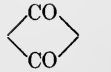
The —NO group in the nitroso dyes (Echtgrün, naphthol green).

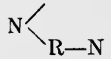
—N=N— in the azo dyes (anilin yellow, chrysoidin, vesuvin, Sudan G and III, alizarin yellow FS, Ponceau, Bordeaux, amaranth, coccinin, orange G, tropaeolin, Biebrich scarlet, congo, benzopurpurin).

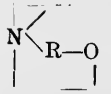

in the rosanilins (malachite green, brilliant green, methyl violet, methyl green, fuchsin, acid fushsin, iodin green anilin blue, alkali blue, water blue, aldehyde green).

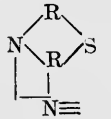

in the rosolic acid dyes (aurins).

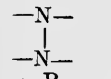

in the phthaleins (eosin, spriteosin, erythrosin, phloxin, rose bengale, rhodamin, gallein, cerulein).

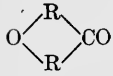

in the anthraquinones (alizarin, purpurin, anthragallol, alizarin blue).

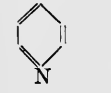

in the indamins (phenylene blue, Bindschedler's green, toluylene blue).


in the indophenols (indophenol blue).


in the thiazins (Lauth's dyes); (Lauth's violet or thionin, methylene blue, methylene red, methylene green).


in the azins (eurhodin, eurhodol, toluylene red, the safranins, Magdala red, mauvein.)


in euxanthinic acid and possibly in galloflavin (jaune indienne).


in the quinolins and acridins (cyanin, quinolin red, quinolin yellow, acridin red, and scarlet).

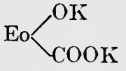
The majority of the anilin dyes are found in the market in the form of salts of the respective staining acids and bases, and it is noteworthy that the two latter, by themselves, are for the most part either colorless or but feebly stained. Triaminotriphenylcarbinol is thus colorless, while its monacid salts are red (fuchsin); phenolphthalein likewise is colorless, but forms red salts with the alkalies; fluorescein is pale yellow, but forms the bright red, fluorescent uranin with alkali, etc. The phenols and nitrophenols, however, such as picric acid, are commonly used as free acids.

During the process of staining the salts of the staining acids or bases are probably decomposed by the animal or vegetable tissue, new compounds resulting between the free staining acids or bases and corresponding combining groups on the part of the various cellular components. The acid nuclear substance of cells thus shows a special affinity for basic dyes, and basic protoplasm for

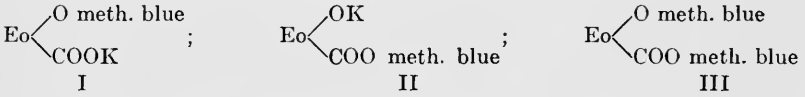
acid dyes. Contrasted with this chemical process of staining is the physical process in which the dye is merely stored in the pores of the tissue. Both must be sharply differentiated, the one from the other, in attempting to draw inferences in reference to the chemical structure on the part of the component parts of a tissue or a cell.

While in former years simple dyes were commonly employed in the clinical laboratory and tissues were stained *successively* if more than one dye was used, it has been shown that it is possible to combine acid dyes with basic dyes in such manner that the acid and basic affinities become more or less completely satisfied. The resulting compounds are spoken of as *neutral dyes*. In these the staining principles of the original components are preserved, and in addition such compounds may show new staining properties which are dependent upon the union of the component dyes. They are accordingly termed *polychrome dyes*.

The credit for having first prepared such neutral dyes belongs to Ehrlich, whose triacid stain was for many years used almost exclusively in the clinical laboratory. At present it has been largely supplanted by eosin-methylene blue and eosin-methylene blue-methylene-azure mixtures. A well-known representative of this order is the eosinate of methylene blue. Eosin is a dibasic acid and can be represented by the formula



Three compounds with methylene blue are thus possible, viz.:



Although the dye has not been analyzed it is thought that formula I or II expresses its constitution. It would thus not be a true neutral dye, but a monacid salt. As a matter of fact, other so-called neutral dyes are, strictly speaking, not neutral. Ehrlich's triacid stain is so called because it was assumed that the three basic radicles of the methyl green were all satisfied by the corresponding acid radicles of acid fuchsin and orange G. The existence of such a triacid salt is, however, impossible in aqueous solutions, even if it could occur theoretically, which in itself is impossible, as methyl green can only form triacid salts with concentrated mineral acids.

It would accordingly be more correct to speak of *neutral mixtures* rather than neutral dyes.

Practically important is the fact that two solutions of neutral mixtures can be directly mixed if they have one component in common, as in the case of Ehrlich's triacid stain, where methyl green is the common component.

While the simple dyes, both basic and acid, are soluble in water,

the neutral dyes are practically insoluble, but soluble in an excess of either the acid or the basic component, and more especially the former. If then an aqueous solution of methyl green is added carefully to an aqueous solution of acid fuchsin, fuchsinate of methyl green is formed at once, but at first remains in solution owing to the presence of an excess of the acid dye. Upon the further addition of methyl green, however, a point is reached where the fuchsinate separates out, and if the amounts of the two components have been carefully determined beforehand the filtrate may be nearly colorless. If then an excess of methyl green is added, a certain amount of the fuchsinate will redissolve; and if the excess be sufficiently great, the entire precipitate will pass into solution.

Aside from an excess of the acid or basic component of the neutral dye its solution can also be brought about in other ways, as with alcohol (notably methyl alcohol), acetone, methylal, etc.

Not all simple dyes are equally well adapted for the preparation of neutral dyes. Of basic dyes the most useful are those which contain the so-called ammonium group, notably methyl green, methylene blue, methylene azure, amethyst blue, and to a certain extent also pyronin and rhodamin; of acid dyes, the readily soluble salts of the polysulphonic acids, such as orange G, acid fuchsin, and narcein, and of the salts of the carbonic acids, eosin. Neutral mixtures may then be prepared which contain two or more component dyes. If it is desired to prepare a tricolor mixture two possibilities suggest themselves, viz., a mixture containing one acid dye and two basic dyes, or one with one basic dye and two acid dyes.

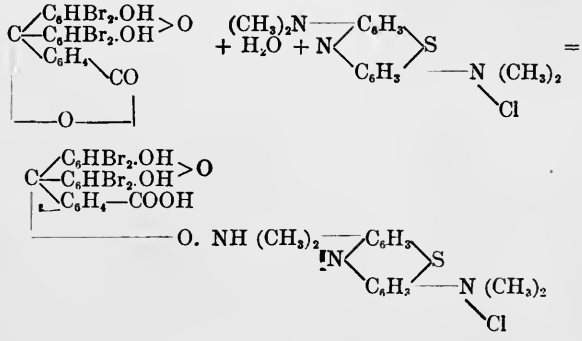
Of the large number of staining mixtures which have been introduced within recent years, and of which many are mere modifications of one another, only a small number of the more common ones will be described at this place, and those only which personal experience has proved to be useful and reliable. Where special mixtures are required for special purposes, they will be found described in their proper connection.

For routine work I should suggest Jenner's method or one of the Romanowsky modifications described below, notably the panoptic mixture of Pappenheim and that of Giemsa, Wilson, Hastings, or Goldhorn. Ehrlich's triacid stain is retained in this edition because it is still used as a routine stain in some laboratories. It is largely of historical interest, however, and less valuable than the others which are mentioned.

METHODS OF STAINING

General Methods.—The Eosinate of Methylene Blue (Jenner).—Equal parts of a 1.2 or 1.25 per cent. *aqueous* solution of eosin and a 1 per cent. *aqueous* solution of methylene blue are mixed in an open

basin and allowed to stand for twenty-four hours. The resulting precipitate—the eosinate of methylene blue—is washed with water, collected on a filter, dried at a moderate temperature, and finely powdered. The reaction which takes place may be represented by the following equation:



The dye can then be stored in bottles and is perfectly stable. For staining purposes a 0.5 per cent. solution in *absolute methyl alcohol* is employed; this can be used at once and keeps indefinitely. I have used this stain as a routine stain for years and can speak definitely of its value. For teaching purposes it has no superior. After a student is thoroughly conversant with blood morphology he may of course use any other.

In preparing the dye I first weigh out the requisite amounts of eosin and methylene blue. The eosin is placed in a mortar or evaporating dish and rubbed into a paste with a small amount of distilled water; more water is then added until all the dye is dissolved. This solution is poured into a large saucepan and diluted to the proper point. The methylene blue is now similarly brought into solution, though with a little more difficulty, as the dye is inclined to be lumpy; it must *all* be dissolved It is poured directly into the eosin solution and the requisite amount of water further added. The mixture is stirred with a rod and left to stand for twenty-four hours.

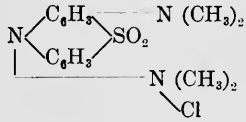
If the proper quantities have been used and *entirely* dissolved, the filtrate is but little colored, in which case not much washing is necessary; if, however, there is a distinct excess of either dye, this must be washed out. The precipitate is dried at a temperature not exceeding 60° C., and is then powdered. The alcoholic solution finally is prepared by rubbing up the dye with the alcohol in a porcelain dish. *Absolute methyl alcohol, free of acid, must be used.* If need be, this is first neutralized with alcoholic caustic alkali.

The blood films (on slides), *which must be prepared without any pressure* (*the spreading slide should really be in contact only with the blood and not with the underlying slide*), are not fixed before staining;

this is accomplished by the absolute alcohol during the staining. The specimens are *flooded* with the stain and after about three minutes washed off with water and dried in the air (blotting is inadmissible). Care should be had during the staining that the preparations are thoroughly covered with the dye, as otherwise some of the stain is apt to become precipitated as the result of evaporation. After drying, the specimens can be examined directly in a drop of cedar oil. With the precautions stated, and by strictly adhering to the method as described, even the beginner can obtain perfect results. For routine purposes I can recommend the stain without reserve. The differentiation is excellent and most extensive (Plates II and VI). The red corpuscles are stained a grayish terra cotta, the nuclei of the leukocytes and nucleated red cells blue, the plaques mauve, the neutrophilic granules a purplish red, the eosinophilic granules bright red, and the mast-cell granules dark violet. Granular degeneration and polychromasia of the red cells is well shown (Plate II). Malarial organisms, bacteria, and filariæ are stained blue. Using this stain, I find it relatively easy to teach even beginners to make a differential leukocyte count with the low power (⅔ B. & L.), while the specimen is as yet wet; the differentiation of the eosinophile from the neutrophile is easier with this stain than with any other of the polychrome dyes with which I am acquainted.

The *May-Grünwald stain,* which is frequently referred to in the German literature, is essentially the same as Jenner's.

The Romanowsky Method.—The history of the Romanowsky method is intimately associated with the study of the minute structure of the malarial organism, in which the presence of a nucleus was first demonstrated by its aid. The dye is essentially an eosin-methylene-blue mixture, the specific staining action of which is, however, not due to the methylene blue *per se,* but to an oxidation product of the methylene blue, viz., methylene azure. This is an amphoteric dye, *i. e.,* a dye of basic constitution with acid properties; it is the sulphone of methylene blue, and has the formula



In making up the stain we do not usually employ solutions of the pure dye, however, which is very expensive, but solutions of methylene blue containing a variable amount of the methylene azure, to which the requisite amount of eosin is added.

The following modifications of the original Romanowsky method are based in principle upon the above considerations:

Hastings' Method.—Three solutions are prepared, viz., (1) a 1 per cent. aqueous solution of eosin (Grübler's water soluble, yellow shade);

(2) a 1 per cent. aqueous solution of methylene blue (Ehrlich's rectified), and (3) a solution of polychrome methylene blue.

The polychrome methylene-blue solution is made according to the following formula: methylene blue (Ehrlich's rectified), 2 grams; sodium carbonate (dry powder), 2 grams; distilled water, 200 c.c. The carbonate is dissolved in hot distilled water and the methylene blue rubbed up in the proportion indicated. The solution is boiled over a free flame or kept on a boiling water bath for ten to fifteen minutes, when 30 to 40 c.c. of water are added for each 100 c.c. to allow for evaporation. The boiling is continued for ten to fifteen minutes longer. The hot solution is poured off from the sediment, and if necessary brought to the 200 c.c. mark by diluting with distilled water, after which it is partially neutralized with dilute acetic acid (12.5 to 20 per cent. solution), using litmus paper as indicator and noting the color above the point of contact with the stain. Hastings points out that it is well to add the acetic acid to one-half of the polychrome-blue solution until a well-marked acid reaction to litmus paper is obtained (6 or 7 c.c. of 12.5 per cent. acid, or 3 or 4 c.c. of the 20 per cent. acid to 100 c.c.), and to mix this neutralized portion with the other half, so as to prevent overneutralization.

The solution should be alkaline in final reaction, since a slight excess of acid destroys the polychrome properties, which cannot be restored by the addition of alkalies. The three solutions are then mixed in the following proportion and in the following order:

Distilled water	1000 c.c.
1 per cent. eosin solution	100 c.c.
Polychrome-blue solution	200 c.c.
1 per cent. methylene-blue solution	70 c.c.

The mixture is stirred. A green, metallic-looking scum appears on the surface and a fine precipitate separates out, which is readily seen by spreading out a drop of the stain on porcelain. To bring about this point it may be necessary to add a little more of the 1 per cent. methylene blue solution, viz., 80 instead of 70 c.c.

The mixture may be filtered at once or after standing for twenty to thirty minutes. The residue is allowed to dry in the air or in the drying oven at a temperature not above 60° C. It is finally pulverized and can be stored in this form. The amounts of the dyes indicated above furnish from 0.7 to 1 gram of the ultimate product.

For staining purposes a 0.25 per cent. solution in *absolute methyl alcohol* is used, which is prepared by rubbing up the dye with the alcohol in a mortar. If successful the solution has a purple plum color.

Care should be had that the alcohol is neutral. Some lots of methyl alcohol show an acidity of 1 to 2 c.c. of $\frac{n}{10}$ alkali for 100 c.c. Such specimens must be neutralized by the addition of 0.05 to 0.1 gram of dry sodium carbonate for 100 c.c.

Previous fixation of the blood specimens is not necessary, as the

alcohol fixes while the staining is going on. The films are covered with the solution and left for one minute, after which they are differentiated by the addition of water until a greenish, metallic-looking scum appears on the surface (15 drops to a slide). This is continued for five minutes, when the preparations are rinsed for two or three seconds in water and immediately dried by blotting. This procedure will answer for all ordinary purposes, and for bringing out the young forms of the malarial parasite, but for the maturer forms it is better to stain for two minutes and to differentiate for ten.

The negative surface of the specimen should be carefully inspected and washed if necessary, to remove any dried stain that may be present and which appears as a thick, greenish coating.

In a properly stained specimen the red cells appear red; in overstained or old specimens light gray or light blue. Polychromatophilia and granular degeneration are well shown. The neutrophilic granules are bright red, the eosinophilic granules eosin colored, and the mast-cell granules dark purplish red. The nuclei of the lymphocytes, large mononuclear leukocytes, and myelocytes are magenta red; those of the polynuclear leukocytes a bluish violet. In some of the lymphocytes and large mononuclear leukocytes Michaelis' granules will be seen. The blood plates are pale blue with red nuclei. The nuclei of the red blood corpuscles are red. The malarial organisms present a blue body with one or more intensely red nuclear structures, varying in size from that of a tiny dot in the youngest forms to a structure which in the microgametocytes fills the entire body of the parasite in the form of a fine reticulum. In the segmenting bodies each segment contains a red nucleus, while the body is blue. In the case of the tertian parasite Schüffner's dots are well marked in the containing red corpuscles. (Plates III, IV, V and XII.)

Wilson's Method.—One hundred c.c. of a 1 per cent. aqueous solution of methylene blue, containing 0.5 per cent. of sodium carbonate, are treated with at least 0.5 gram of freshly precipitated oxide of silver. This is prepared by dissolving 2 grams of silver nitrate in 15 c.c. of distilled water and precipitating the silver oxide by the addition of 260 c.c. of saturated lime water, the oxide being then dried at a temperature of about 90° C. After adding the silver the methylene blue solution is boiled for twenty minutes, then one-third of the fluid is removed; after twenty minutes further, one-half is taken away, while the remainder is left for the balance of an hour. The three portions are then reunited and the volume brought up to the original 100 c.c. mark with distilled water. After standing for a half hour, 100 c.c. of a 0.5 per cent. solution of eosin are added, the fluid well stirred, and then allowed to stand for an hour, when the precipitated dye is collected on a filter, washed several times with normal salt solution, and allowed to dry at a temperature not higher than 60° C. The pulverized product is stored in a dry bottle. For staining

purposes a 0.4 per cent. solution in absolute and neutral methyl alcohol (see Hastings' stain) is used. The blood films require no previous fixation; they are covered with from 5 to 10 drops (according to size) of the dye, which is left in concentrated form for one minute; after this as many drops of water are added and the diluted stain allowed to remain for four minutes longer. The specimens are then washed and blotted at once. The coloring of the various elements is the same as with Hastings' stain.

Giemsa's Method.—Giemsa's stain[1] has the following composition:

Azure II (azure plus methylene blue āā)	3.0
Eosin (B. A.)	0.8
Glycerin (Merck, C. P.)	250.0
Methyl alcohol (Kahlbaum I)	250.0

It is prepared by rubbing up the dyes in the absolute alcohol and then adding the glycerin. The blood films are fixed for a minute in absolute methyl alcohol and then stained for five minutes in a mixture of 14 drops of the dye to 10 c.c. of distilled water, which is always freshly prepared; a trace of sodium carbonate may be added to the water to intensify the basic color. After washing in water the films are blotted and are then ready for examination. The various elements are stained as with the methods already described.

Goldhorn's Method.—The blood smears are fixed with pure methyl alcohol for fifteen seconds, washed in running water, stained for thirty seconds in a 1 per cent. aqueous solution of eosin, washed, stained for one minute in Goldhorn's polychrome methylene blue, again washed, and dried in the air.

The polychrome methylene blue is prepared as follows: 2 grams of methylene blue and 4 grams of lithium carbonate are dissolved in 300 c.c. of warm water. The solution is heated in a porcelain dish on a boiling water bath for fifteen minutes, then poured into a glass-stoppered bottle and set aside for several days. The strongly alkaline reaction is finally reduced to a slight grade of alkalinity by the careful addition of 4 to 5 per cent. acetic acid solution (test with litmus paper). The method gives excellent results.

Panoptic Stain of Pappenheim.—Pappenheim has suggested the following procedure which combines the advantages of the eosinate of methylene blue with those of the Romanowsky stain. The blood smears are first stained for three minutes with Jenner's stain and differentiated for one minute by adding to the stain on the slide an equal volume of water. The diluted stain is poured off and replaced with aqueous Giemsa's solution, using 15 drops, *i. e.*, 3 c.c. diluted with 10 c.c. of distilled water. The reagent is left on the specimen for fifteen minutes, when it is washed in water, blotted dry (no flame), and may then be mounted in neutral Canada balsam, or examined directly in oil. The results are said to be almost uniformly good.

[1] This is the so-called second formula of Giemsa.

More recently Pappenheim has suggested that the procedure may be simplified as follows:

1.5 c.c. of Giemsa's solution (formula given above) is mixed with 1 c.c. of Jenner's stain and 0.2 c.c. of acetone (though the last is not essential). The smear is stained (fixed) with this solution, then differentiated by adding an equal quantity of distilled water and leaving this for twelve to fourteen minutes, when the specimen is rinsed off and allowed to dry. If there should be overstaining the dry film is briefly treated with ethyl alcohol (absolute) which is allowed to drain off by the aid of filter paper.

Ehrlich's Triacid Stain.—The preparation of a reliable triacid stain, according to Ehrlich, presupposes the use of chemically pure dyes, such as those prepared by the Actiengesellschaft für Anilinfarbstoffe of Berlin. Saturated aqueous solutions of orange G, acid fuchsin, and methyl green are first prepared and allowed to clear by standing for at least one week. It is essential that these solutions should be perfectly clear, and it is well in measuring off the requisite quantities to remove the supernatant portion with a pipette.

The various components are mixed in a clean bottle, making use of the same measuring glass, and without washing between the addition of the individual components. These are taken in the succession shown below, and after adding the methyl green the mixture is thoroughly stirred until the remaining portion of alcohol and glycerin has been added.

Orange G solution	13.0 to 14.0 c.c.
Acid fuchsin solution	6.0 to 7.0 c.c.
Distilled water	15.0 c.c.
Absolute alcohol	15.0 c.c.
Methyl-green solution	12.5 c.c.
Absolute alcohol	10.0 c c.
Glycerin	10.0 c.c.

The solution is ready for use at once and does not deteriorate with age.

In order to obtain the best results, it is practically necessary to fix the blood films by heat; fixation by absolute alcohol or a mixture of equal parts of absolute alcohol and ether does not furnish constant results, and only too often leaves the neutrophilic granules unstained or imperfectly stained. Brief fixation at a high temperature (140° C. for thirty to forty-five seconds, using xylol droplets as indicator of the temperature, as suggested above, p. 63) furnishes better results than the lower temperatures originally advised by Ehrlich, as the difference in color between the neutrophilic granules and the eosinophilic granules is brought out more prominently. The blood specimens are stained about five minutes, then washed in water, dried (by blotting, if desired), and examined as usual.

In properly stained specimens the eosinophilic granules present a

copper or a yellowish-red color, while the neutrophilic granules are violet. The mast-cell granules remain colorless and appear as round vacuoles in a bluish-green background. The nuclei of the leukocytes present a greenish color and are not well stained. The red cells in properly heated specimens are orange; if the temperature was too high they are yellow, and it will be found that their structure has suffered as a consequence. If the temperature has been too low the red cells take on the fuchsin. The nuclei of the normoblasts are intensely stained; the older nuclei appear black; megaloblastic nuclei, on the other hand, are rather feebly colored, and in some specimens, indeed, the inexperienced will at first sight not discern any nucleus. Granular degeneration is not shown and polychromatophilia cannot be well demonstrated. Malarial organisms are imperfectly shown. The differentiation with the triacid is thus markedly less than in the case of the other dyes described above. This is owing to the peculiar character of the methyl green, which is a specific nuclear dye. To counteract some of these deficiencies, Ehrlich has suggested to stain the preparations for a few seconds with an aqueous solution of methylene blue first, and to stain with the triacid afterward. This improves the pictures somewhat, but it is not wholly satisfactory.

DEMONSTRATION OF IODOPHILIA

Cover-glass specimens are prepared as usual; after drying in the air they are placed in a small jar containing a few crystals of iodin. After several minutes the films assume a dark brown color, when they are mounted in a drop of a saturated solution of levulose and examined with an oil-immersion lens. The red corpuscles are stained light yellow, while the leukocytes are almost colorless. All glycogen granules, whether contained in leukocytes or free in the blood, are stained a mahogany.

This method furnishes better results than the older method of staining with a solution composed of 1 gram of iodin and 3 grams of potassium iodide in 100 grams of a concentrated solution of mucilage (1 part of Lugol's solution to 100 parts of a thick mucilage.)

ENUMERATION OF THE CORPUSCLES OF THE BLOOD

Method of Thoma (*Author's Modification*[1]).—The instrument consists of two diluting pipettes and a counting chamber (Fig. 7). The latter is ruled into 100 large squares (*A*, *A*, *A*), each occupying an area of $\frac{1}{25}$ sq. mm. (Fig. 8). They are separated from one another by double guide lines (*a b*, *a b*) with an intervening distance of $\frac{1}{20}$ mm. Where the horizontal and vertical lines intersect small squares

[1] The Simon counting chamber can be procured from Ernst Leitz & Co., New York.

(*a*, *a*, *a*) result, 100 in number, which accordingly have an area of $\frac{1}{400}$ sq. mm. each. The large squares are thus bounded by rectangles (*b*, *b*, *b*), measuring $\frac{1}{20}$ mm. in width by $\frac{4}{20}$ mm. in length, representing an area of $\frac{1}{100}$ sq. mm.

As the little platform (*f*) carrying the ruling is exactly $\frac{1}{10}$ mm. lower than the outside glass plate (*e*), each large square represents the base of a cube the contents of which are $\frac{1}{25} \times \frac{1}{10} = \frac{1}{250}$ c.mm.; each small square similarly corresponds to $\frac{1}{400} \times \frac{1}{10} = \frac{1}{4000}$ c.mm. and each rectangle to $\frac{1}{100} \times \frac{1}{10} = \frac{1}{1000}$ c.mm.

1. Enumeration of the Leukocytes.—The drop of blood from which the count is to be made must be procured with a considerable amount of care. The puncture, above all, should be sufficiently free to insure a ready flow of blood without any special degree of pressure. Where pressure is used, the absolute count will of necessity be wrong and need not be attempted. Equally important is the point of puncture. Generally speaking, the ear is preferable to the finger. If it is seen, however, that the ear is congested, from the fact that the patient has been lying on that side, or from other reasons, the normal ear should be chosen, or the finger. In small children I take the big toe. A small lance is better than a

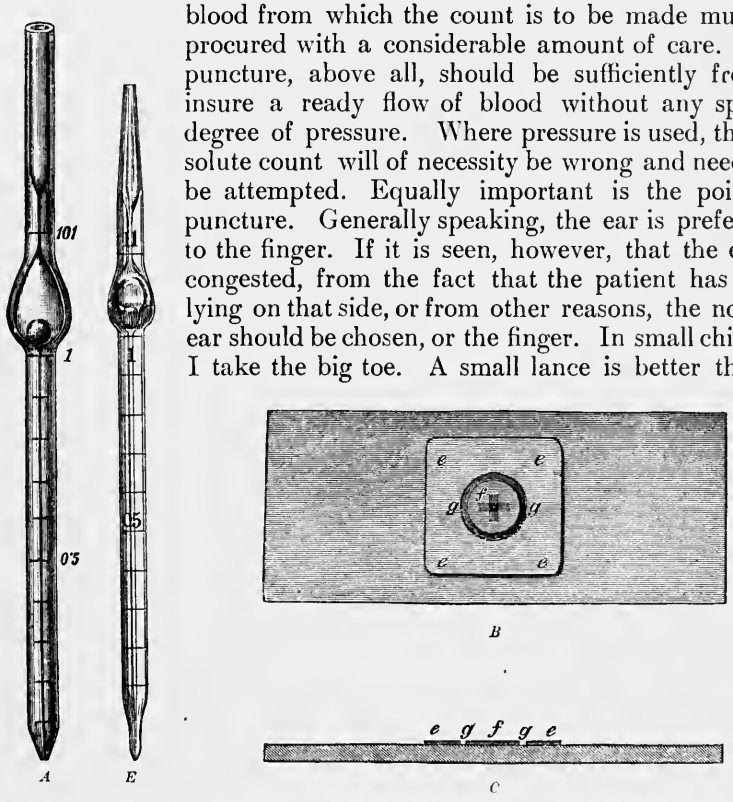


FIG. 7.—Simon blood-counting apparatus. *A* and *E*, red and white diluting pipette, respectively; *B*, counting chamber, seen from above; *C*, profile of counting chamber.

needle. In any event the instrument, as well as the skin, is cleansed with alcohol and dried; the first drop or two are wiped away and the blood then drawn into the 1 to 10 diluting pipette to the mark 1, and after carefully wiping the end is immediately mixed with a 5 per cent. solution of glacial acetic acid by drawing up the mixture to the 11 mark. 0.3 to 0.5 per cent. solutions of

acetic acid, which are recommended in some text-books, are entirely too weak to cause the complete destruction of the red cells. If desired one may color the acid by the addition of a trace of methyl green (0.03 gm.) or vesuvin (0.03 gm. per 250 c.c. of acid). The advantage is that the leukocytes stand out more distinctly when colored, and that confusion of bottles is less apt to occur by having the diluent for the leukocytes different in color from that for the red cells. The rubber tube of the pipette is then detached, both ends of the pipette closed

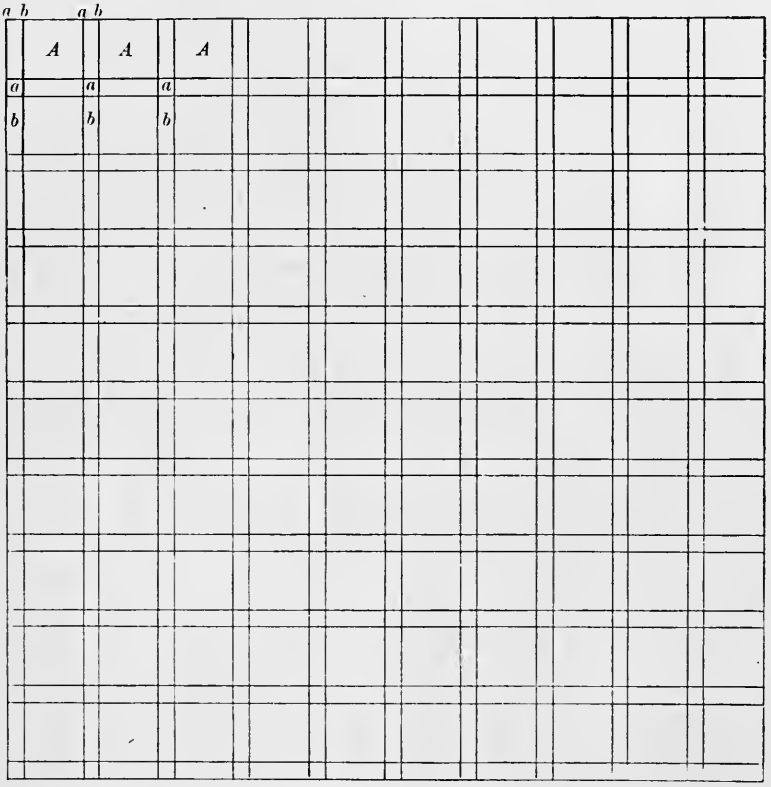


Fig. 8.—Simon's counting chamber.

with the thumb and middle finger, and blood and diluent well agitated. If the specimen is secured at the patient's house and must be transported to the laboratory, provision should be made that the tube does not run out on the way. To obviate this, the point may be plunged into a small cork, or a rubber band is passed over both openings. Before a mount is made the specimen should be vigorously shaken for several minutes, so as to bring about an even distribution of the cells. The contents of the stem of the pipette are then blown out, as they

merely represent the diluting fiuid. After this a drop of the diluted blood is placed upon the platform of the counting slide, and one of the cover-glasses which accompany the instrument adjusted in such a way as to exclude bubbles of air. The size of the drop should be such that, when the cover-glass is in place, it does not run over into the moat (*g*) surrounding the circular platform, or even project over the sides. Türck advises that a *tiny* droplet of the pure diluting fluid be placed upon the plate *e* before the diluted blood is placed upon the counting platform. If cover and slide have been previously scrupulously cleansed and slight pressure is now made upon the cover where it overlies the plate *e*, Newton's colored rings will become visible—a sign that a successful mount has been made. The slide is set aside for a few minutes, so that the corpuscles settle down, when it is examined with a low power (B. & L. $\frac{2}{3}$); a higher magnification is not only unnecessary, but even undesirable. With the low power a count can be made in from six to ten minutes. The red corpuscles, of course, have been destroyed and do not appear in the field. A mechanical stage is unnecessary. Starting with the top row of large squares at the left corner (Fig. 6) the total number of leukocytes in the 100 large squares is carefully counted. This number divided by 100 gives the average number of leukocytes for *one* large square. As the cubic contents of each large square are $\frac{1}{250}$ c.mm., it is necessary to multiply the average number of leukocytes in one square by 250 in order to find the number for 1 c.mm. of *diluted* blood, and this by the degree of dilution (in the above instance by 10) to find the number for 1 c.mm. of diluted blood.

Example.—Total number of leukocytes counted in the 100 large squares = 400; hence $\frac{400}{100}$, viz., 4 = number of leukocytes in a single square, *i. e.*, in $\frac{1}{250}$ c.mm. of diluted blood; hence $250 \times 4 = 1000$, the number of leukocytes in 1 c.mm. of diluted blood, and $1000 \times 10 = 10{,}000$ the number in 1 c.mm. of non-diluted blood.

When counting the cells, note should only be taken of such that lie *within* the squares or upon the upper and left boundary lines; cells upon the right and lower lines, as well as cells which only touch the lines, but manifestly lie outside the squares, should be omitted.

In the above instance a dilution of 1 to 10 has been advocated. This may be used as a matter of routine. If a high grade of leukocytosis is anticipated a dilution of 1 to 20 will be found more convenient. If desired, higher dilutions even may be used, in which case the red pipette, permitting of a dilution of 1 to 100 or more, may be employed.

2. **Enumeration of the Red Cells.**—The simplest diluent and one that is perfectly satisfactory for counting the red cells is 0.9 per cent. saline to which a trace of methyl violet or toluidin blue may be added (0.1 gm. per 250 c.c. saline) in order to distinguish the leukocytes from the red cells.

The diluent which has otherwise been longest in use is Toison's solution; its composition is the following:

Sodium chloride	1.0
Sodium sulphate	8.0
Neutral glycerin	30.0
Distilled water	160.0
Methyl violet (5 B.)	0.025

Ringer-Locke's solution is of course also to be recommended. Its composition is the following:

Sodium chloride	6.0
Calcium chloride	0.2
Potassium chloride	0.4
Sodium bicarbonate	0.2
Dextrose	1.0
Distilled water	1000.0

To prevent the development of moulds the solutions should contain about 1 pro mille of thymol.

The blood is diluted 100 times by filling the red pipette to the mark 1 with blood and drawing up the diluent to the mark 101, or if the patient does not appear to be especially anemic by taking only half a volume of blood and diluting as before to the 101 mark, which would give a dilution of 1: 200

After mixing the diluent and blood thoroughly and blowing out the contents of the stem of the pipette which contains diluting fluid only, a drop is mounted as described in the case of the leukocytes. All the red corpuscles are then counted—in the 100 *small* squares, if no marked degree of anemia exists, or in 100 or more *rectangles* if the corpuscles are greatly diminished. The calculation is made as follows, bearing in mind the cubic contents, corresponding to the small square and the rectangle, viz., $\frac{1}{4000}$ and $\frac{1}{1000}$ c.mm., respectively:

Example 1.—Number of red cells in 100 small squares = 1000; in 1, therefore, 10, viz., in $\frac{1}{4000}$ c.mm.; in 1 c.mm. of diluted blood $4000 \times 10 = 40{,}000$, and in 1 c.mm. of non-diluted blood $40{,}000 \times 100 = 4{,}000{,}000$.

Example 2.—Number of red cells in 100 rectangles = 800; in 1 rectangle, therefore, 8, *i. e.*, in $\frac{1}{1000}$ c.mm.; in 1 c.mm. of diluted blood, hence $8 \times 1000 = 8000$, and in 1 c.mm. of non-diluted blood, $8000 \times 100 = 800{,}000$.

If for any reason a larger area is to be counted, this can, of course, be readily done by going over a larger number of rectangles, or by combining small squares and rectangles, due allowance being made for the cubic contents of the ground covered.

Cleaning of the Apparatus.—After use the apparatus must be carefully cleansed. The pipette is washed out with the diluting fluid, then with water, next with absolute alcohol, and finally with ether. The washing will be facilitated by slipping the rubber tube over the long arm of the pipette and blowing the contents of the bulb out of

the short arm. In laboratories which are equipped with a suction pump this may be conveniently employed; the entire process then occupies only two or three minutes.

The counting chamber is washed with water only; alcohol and ether dissolve the substance with which the platform is cemented to the slide.

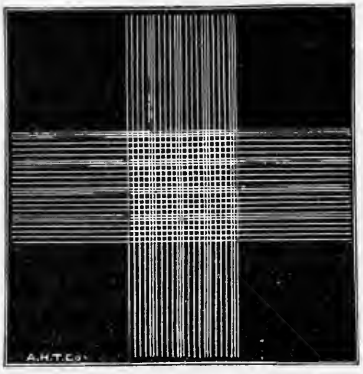


Fig. 9.—Thoma.

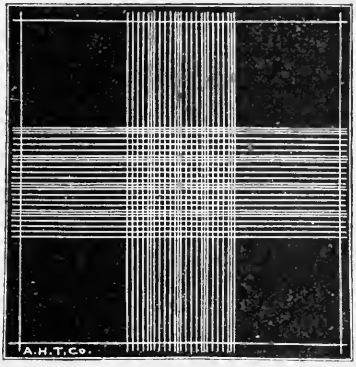


Fig. 10.—Zappert.

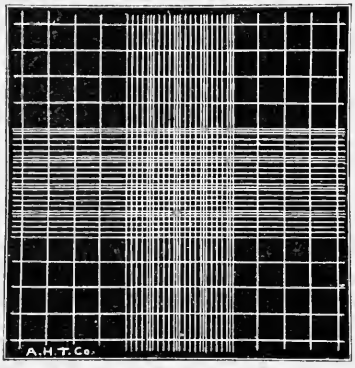


Fig. 11.—Neubauer.

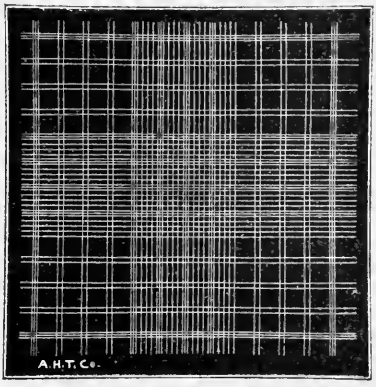


Fig. 12.—Türck.

Blood-counting chambers.

Other counting chambers are also in existence. The form of the ruling of various models is shown in the accompanying figures (Figs. 9 to 12). They are used in the same manner as my own. The calculation in each case depends upon the number of squares counted, the corresponding cubic contents, and the degree of dilution.

Quite convenient are the double counting chambers designed by Bürker and marketed by Leitz, in which two rulings have been mounted on one slide, the one intended for the white, the other for the red count (Figs. 13 and 14).

Planned on similar lines are the double counters marketed by Arthur Thomas & Co., of Philadelphia (made by M. Levy), and illustrated in Fig. 15. The essential points of difference are these: in the original Bürker type the ruled pieces of glass not only are cemented to a slide, but two unruled pieces of glass are

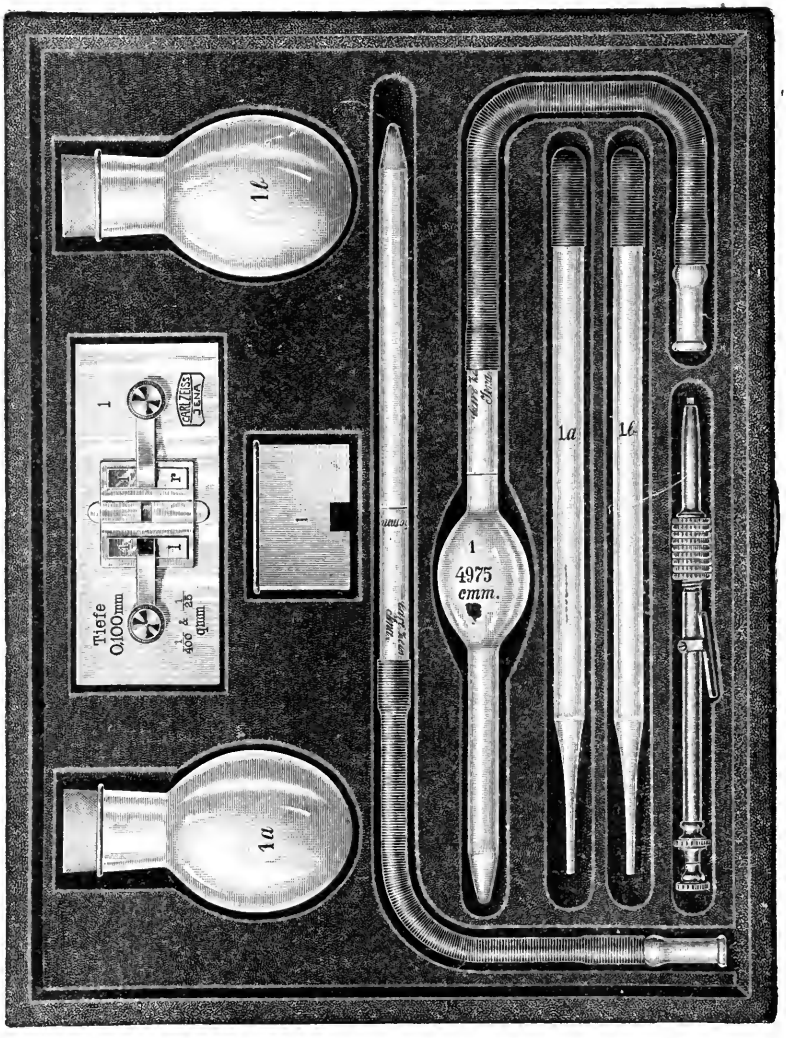


Fig. 13.—Bürker hemocytometer.

placed on either side to obtain the necessary depth, while in the Levy instruments a rectangular depression is cut into the slide itself across its entire width, in the middle of which the ruled strips are fastened. Any of the well-known rulings may be obtained in this form.

Differential Enumeration of the Leukocytes.—The differential enumeration of the leukocytes is usually made in dried and stained specimens. Beginners would do well to practise this at first with the oil-immersion lens. After a little practice, however, it is more convenient and less time consuming to make the count with the *lowest* power of the usual microscopic outfit (B. & L. $\frac{2}{3}$). In order to obtain the proper degree of refraction the stained surface of the specimen is wetted with water, or it is covered with immersion oil. The condenser is thrown out and a moderately subdued light obtained by using the flat mirror. The best results are obtained with speci-

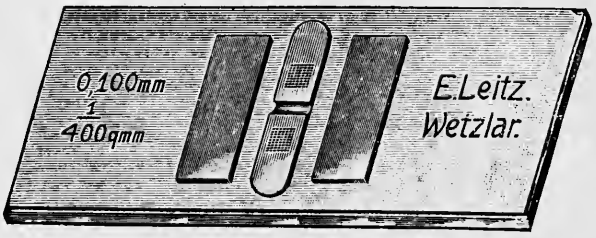


FIG. 14.—Bürker's counting chamber.

mens stained with an eosin-methylene-blue mixture (Jenner's stain), as the differentiation of the eosinophiles from the neutrophiles is thus best effected. The small mononuclears appear as small, well-stained blue little bodies; the large monos are of a paler blue and larger; the polynuclear neutrophiles show a multiple or multiform blue nucleus in a pinkish background; the eosinophiles attract attention at once by the luminous red surrounding the blue nucleus, while the mast cells can also be distinguished without difficulty; their granules are seen as tiny black specks on a pale blue surface—the nucleus.

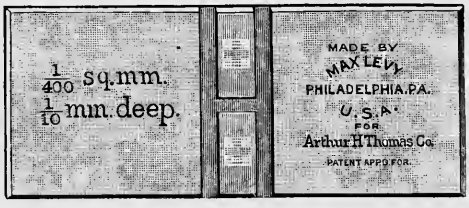


FIG. 15.—Thomas-Levy hemocytometer.

Myelocytes can also be recognized with the low power, but in counting leukemic blood it is probably better to resort to the oil immersion and to cut down the field by placing a small diaphragm of paper upon the little stage in the interior of the ocular.

With the use of the low power in routine work, a differential count of 300 cells can readily be made in ten minutes. In my personal work I take the freshly stained slide, wiped dry on the

bottom, but still wet on top, to the microscope and have my count finished before the specimen has had time to become dry. The idea in counting is to go over a large number of cells; for ordinary purposes not less than 300, to classify these, and finally to calculate the percentages. The larger the number counted, the more accurate, of course, will be the result. The cells may be charted as shown below:

S. M. (small mononuclear leukocytes): 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 = 45

L. M. (large mononuclear leukocytes): 𝍸 𝍸 𝍸 = 15

P. (polynuclear neutrophiles): 𝍸 = 155

E. (eosinophiles): 𝍸 = 5

M. (mast-cells): // = 2

222

Result: Total number of cells counted, 222, of which:

small monos., $\frac{45\times100}{222}=20.2$ per cent

large monos., $\frac{15\times100}{222}=6.7$ "

polys., $\frac{155\times100}{222}=69.8$ "

eosins., $\frac{5\times100}{222}=2.2$ "

mast, $\frac{2\times100}{222}=0.9$ "

Or one may count a large number of the cells in one's head and put them down as follows:

Small.	Large.	Polys.	Eosins.	Mast.
35	10	64	1	1
28	6	50	—	—
30	14	61	—	—
—	—	—	—	—
93	30	175	1	1=300

Small = 31.0 per cent.
Large = 10.0 "
Polys. = 58.3 "
Eosin. = 0.3 "
Mast = 0.3 "

While making a differential count it is well to keep note of the time, as it is often possible in this way to form a fair idea of the *actual* number of the leukocytes without an absolute count. This, of course, requires a certain amount of experience in the preparation of the smears, which should be uniformly of nearly the same thickness.

After one has then learned by control how many leukocytes in a blood smear, observed within a certain length of time, may be considered as normal, it is not difficult to judge the grade of a hyperleukocytosis by the increase in number noted within the same length of time. Everyone must here work out his personal equation.

Enumeration of the Plaques.—For this purpose the method of Brodie and Russell has been advocated. The method is an indirect one. First, the red corpuscles are counted in the usual manner. A drop of the staining fluid, composed of equal parts of a 2 per cent. solution of common salt and a saturated solution of dahlia in glycerin, is then placed upon the finger, when this is punctured through the drop and the blood allowed to mix with the reagent. In this mixture the ratio between the plaques and the red corpuscles is ascertained, and the total number of plaques contained in 1 c.mm. of blood determined by calculation. The plaques are stained the color of dahlia and can readily be counted. Rapid work is essential, as the staining fluid soon attacks the red corpuscles.

Other writers determine the ratio of plaques to red cells in smears and then calculate their number after an absolute red-cell count. Jenner's stain or any one of the methylene-azure mixtures (Hastings', Giemsa's, Wright's) will answer the purpose.

The Arneth Count.—Whereas Arneth has recommended the percentage enumeration of the neutrophiles on the basis of the number of nuclear segments (see p. 38), Pappenheim has pointed out that this is apt to lead to erroneous results, and recommends the mere division of the neutrophilic cell group into myelocytes, metamyelocytes, and leukocytes, which will indicate the degree of bone-marrow reaction by whatever stimulus may be at work.

The Hematocrit.—The use of the hematocrit for counting the red blood corpuscles has been repeatedly advocated, but has not met with favor. The method is inapplicable whenever there is any material variation in the size and form of the red corpuscles and whenever the number of the leukocytes is greatly increased. This means that the method cannot be employed in the majority of cases in which we are especially interested in the blood count. If, however, it is desired to ascertain the volume of the red corpuscles in relation to the amount of plasma, the instrument will furnish satisfactory results. A centrifuge run by electricity is practically a necessity; in this way alone is it possible to maintain the proper rate and uniformity of speed. Hand centrifuges are totally inadequate, and with instruments driven by water power it is impossible to attain a sufficient rate of speed for this purpose. An apparatus like the one pictured in the accompanying illustration (Fig. 16) answers the purpose best. It is connected with the street current or with a small battery, a rheostat being interposed to control the current and the rate of speed. At the same time a speed indicator can be attached which strikes a bell for

every 100 revolutions. For the hematocrit a speed of 8000 to 10,000 revolutions per minute is required.

The hematocrit which has met with most favor in the United States is that of Daland (Figs. 17, 18, 19). It consists of a metallic frame which carries two glass tubes measuring 50 mm. in length and 0.5 mm. in diameter. Each tube bears a scale ranging from

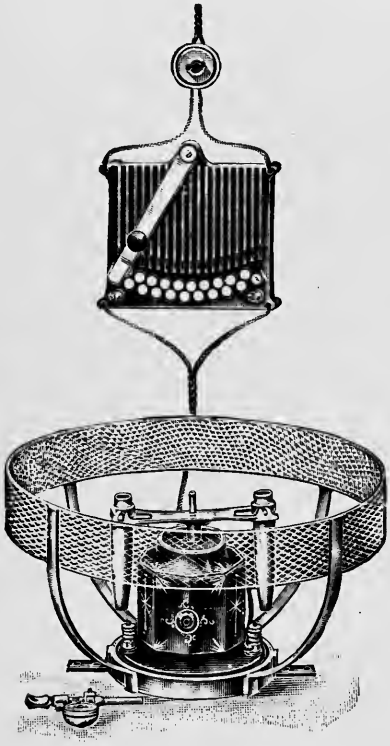


FIG. 16.—Improved electric hematocrit, with fender, rheostat, and speed indicator. The hematocrit attachment replaces the urine tubes seen in the revolving armature.

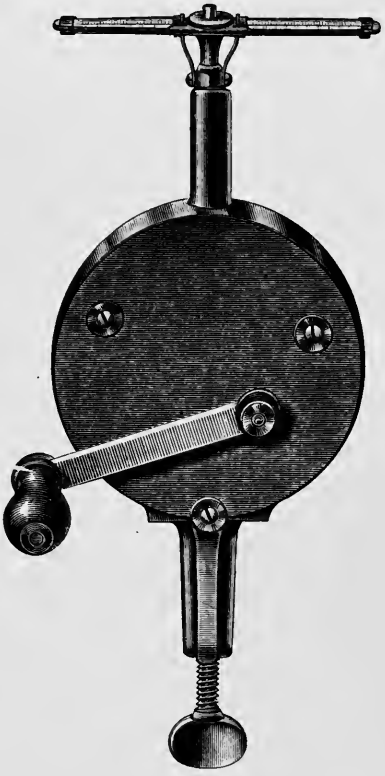


FIG. 17.—Daland's hematocrit.

0 to 100, the individual divisions of which are rendered more easily visible by a magnifying lens front. In the frame the outer end of each tube fits into a small depression, the bottom of which is covered with thin rubber; the inner ends are held in position by springs. The instrument is screwed to a firm table and is oiled daily when in use.

If the patient is directly available, undiluted blood is used. The finger is washed with soap and water and alcohol, as usual, and is

freely punctured. A small rubber tube is then slipped over the end of one of the hematocrit tubes, which is completely filled by suction. The bevelled end of the tube is quickly covered with the finger, which has been previously lubricated with a little vaselin; the rubber tube is disconnected, and the glass tube immediately fixed in the one compartment of the frame. Its mate is rapidly placed on the opposite side and the instrument rotated at a speed of from 8000 to 10,000 revolutions per minute for three minutes, when the volume is read off. In normal individuals the volume of the red

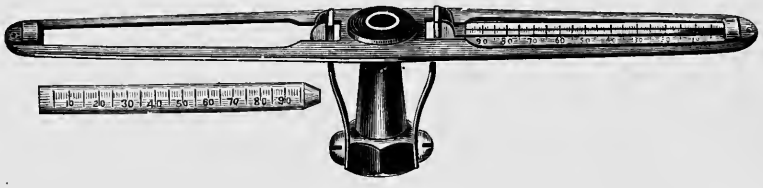


FIG. 18.—Daland's hematocrit.

corpuscles is approximately 50 per cent., so that in a given case a proportionate expression of the percentage of corpuscles, as compared with the normal, can be obtained by multiplying the figure on the scale by 2.

If the patient is not directly available, the blood is diluted with an equal volume of a 2.5 per cent. solution of potassium bichromate, as proposed by Daland. This can be done with the pipette which accompanies the Thoma-Zeiss blood counter. In the case of the red pipette the capillary tube is filled with blood to the mark 1, then a small air bubble is drawn in, followed by another tube length of blood. Three or four volumes of blood are obtained in this way

FIG. 19.—Daland's hematocrit tube.

and diluted at once with an equal quantity of the bichromate solution. In the case of the white pipette a single tube length of blood and the diluent is sufficient. Blood and diluent are thoroughly mixed, care being had not to include any air bubbles. In this form the blood is carried to the laboratory, where both tubes are filled by allowing the drops to flow in from the point of the pipette. To obtain the percentage volume, the resultant figure is in this case, of course, multiplied by 4.

In the case of normal blood it has been ascertained that 1 per cent. by volume, as read off from the scale, corresponds to almost

100,000 red corpuscles per c.mm.; to obtain the total number of red cells per c.mm., it is hence only necessary to add five ciphers to the percentage indicated on the scale.

Example.—Undiluted blood was used; the reading on the scale was 45. The volume per cent. of the red corpuscles would hence be 90, and the number of red cells per c.mm., 4,500,000.

But, as I have pointed out, this calculation presupposes that the size and form of the red cells are practically normal, and that the leukocytes are not materially increased.

With normal blood the leukocytes appear only as a narrow, indistinct, milky band at the central end of the column of red cells, which with a material increase of the leukocytes becomes more marked and reaches its greatest extent in cases of leukemia.

Aspelin has suggested that with a suitable modification of the Daland apparatus quite accurate leukocyte counts can be obtained by centrifugation; but bearing in mind the variations in the size of the different leukocytes and the varying degree to which the different forms take part in the production of the different types of hyperleukocytosis, it is evident that still less is to be anticipated from the centrifugal method in this direction than in the case of the red cells.

Volume Index.—The term *volume index* has been introduced by Capps to designate the relation existing between the volume of red cells determined by centrifugation (see above) and their number. If both are normal the ratio $\frac{\text{volume (100 per cent.)}}{\text{number (100 per cent.)}} = 1$ (0.99 average of 10 normal individuals). Under pathological conditions the index may be increased or diminished. In 29 cases of pernicious anemia it was high during the active stage of the disease, ranging from 1.05 to 2. During periods of improvement it steadily fell, while in periods of decline it rose. In chronic secondary anemia of moderate intensity normal values were the rule; in a few they were low. In acute secondary anemia (sepsis, hemorrhage) the index may be low (0.72); so also in chlorosis of the severer type. In a few cases of chronic severe secondary anemia (as in uncinariasis) Capps found the index high. Analogous results have been obtained by Wroth.

ESTIMATION OF HEMOGLOBIN

Hemoglobinometers.—While it is usually possible to form a fairly clear idea of the degree of anemia by direct inspection of the patient, the appearance of the mucous surfaces, etc., it is often desirable to obtain more definite information, and, above all, a numerical expression of the extent of the anemia. This is especially important in the diagnosis of certain forms of anemia, in which the "color index" plays an important part—*i. e.*, the ratio between the percent-

age of hemoglobin and the percentage of the red corpuscles, as compared with the normal. To this end special instruments have been devised, which are termed *hemoglobinometers* or *hemometers*. Of the various forms which are now in the market, the hemoglobinometer of Dare is probably the best, and has largely replaced the older instrument of v. Fleischl, which for many years was the standard. It is more exact and more convenient. Miescher's modification of the Fleischl instrument is possibly still more accurate, but too costly for general adoption. The little instrument of Gowers, in the modification of Sahli, when obtained from a reliable source will also furnish good results. Unfortunately many of those which have been placed on sale are worthless. The Talquist method is warmly recommended by Cabot, and may be used to advantage in routine work by the general practitioner; for exact work it is insufficient.

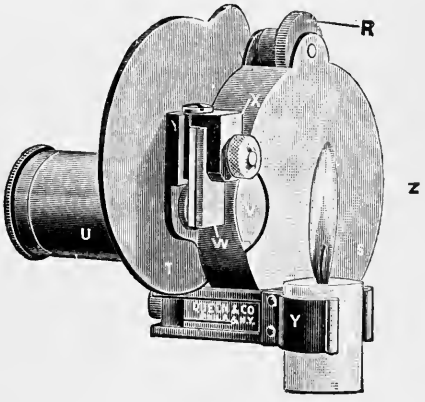


FIG 20.—Dare's hemoglobinometer.

Dare's Hemoglobinometer.—The essential parts of Dare's hemoglobinometer (Fig. 20) are an automatic pipette for collecting the blood (Fig. 21) and a graduated color scale (Fig. 22) to measure the corresponding percentage of hemoglobin. This latter reads from 10 to 120, the 100 mark corresponding to the color of a solution of

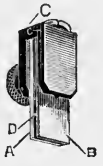


FIG. 21.—Automatic pipette.

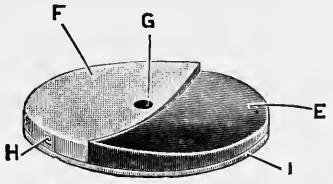


FIG. 22.—Graduated color scale.

13.77 grams of hemoglobin in 100 c.c. of serum. The various shades of color corresponding to the scale are obtained by rotation of a prismatic glass semicircle tinted with the golden purple of Cassius (Fig. 22, *E*), which is secured to a thin white glass disk (*I*). The numerical scale is placed on the edge of a corresponding semicircle (*H*) of thick white glass (*F*). This part of the apparatus is inclosed in a dust-proof hard-rubber case, and is rotated from the outside by the aid of a rubber-covered roller which runs on the edge of the disk

and is turned by a milled wheel at *R* (Fig. 20). In the rubber case is a little circular window through which the color of the prism is viewed by means of a small telescoping camera tube (Fig. 23, *N*), provided with a magnifying lens of low power. The color aperture represents a surface about equal to 3 per cent. of the color scale. Looking through the tube a corresponding window will be seen side by side with the one through which the color scale is visible. In front of this the blood pipette is secured. The essential part of this is an oblong plate of white glass (Fig. 21, *A*), into the end of which a depressed surface of measured depth is ground, the floor being exactly parallel to the plane surface of the glass. This depression forms a capillary chamber (*D*) when the transparent glass plate (*B*) is firmly clamped upon it by the pipette clamp *C;* it is filled by capillary attraction when either of the three free edges is touched to the blood drop. The pipette is held in position on the stage of the instrument by guides which run in grooves on the lower part of the clamp. The plate of white glass is toward the light.

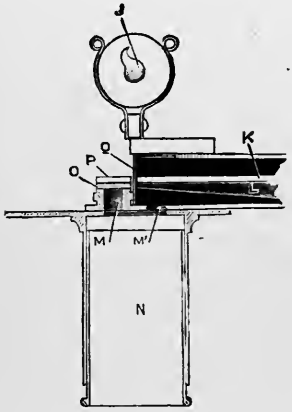


FIG. 23.—Horizontal section of Dare's hemoglobinometer (on a level with centre of comparison apertures): *J*, candle; *K*, white glass disk of color prism; *L*, color prism; *M*, aperture through which color of the blood film is viewed; *M'*, aperture through which the illuminated color prism is viewed; *N*, camera tube; *O*, transparent glass of pipette; *P*, white glass of pipette.

The camera tube screws into a movable shutter (Fig. 20); when this is swung outward the two apertures become visible through which the blood and the color scale are viewed.

In front of the pipette a candle is clamped in such a position that both the blood and the color scale are equally illuminated.

METHOD OF USE.—As the comparison of the color of the blood with that of the color scale should be made as soon after filling the pipette as possible, the apparatus is prepared for use beforehand by screwing the camera tube into place and adjusting the candle; this should be at such a level that the blue flame of the candle is below the color aperture, care being taken to have the wick of proper length (half-inch) and not charred at the tip. Curved or eccentric wicks should be turned so that the intensity of light in a vertical position is midway between the two color apertures.

The glass plates of the pipette having been thoroughly polished and refastened in the clamp, the finger or ear is freely punctured as usual and the capillary space of the pipette filled with the blood, by holding one of the three edges horizontally to the drop (Fig. 24). Any blood adhering to the flat surfaces of the glass plates is wiped away

and the pipette placed in position. The candle is lighted, the shutter thrown out, the camera tube focussed, and the color of the blood (on the left) compared with the color scale (on the right). The two are matched by rotating the color disk by means of the milled wheel, which should be done in an abrupt manner, and frequently resting the eye. To this end the shutter is dropped and thrown out again as the case may be. The examination need not be conducted in a darkened room, but it is important to turn the instrument toward a dark background, so as to eliminate direct or reflected light. The reading is indicated at the bevelled edge of the rectangular opening on the side of the case; the figure immediately beneath this represents the percentage of hemoglobin. Immediately after use the two glass plates of the pipette are cleansed with water and a little acid alcohol, dried, and again replaced. Further details in regard to technique accompany the instrument.

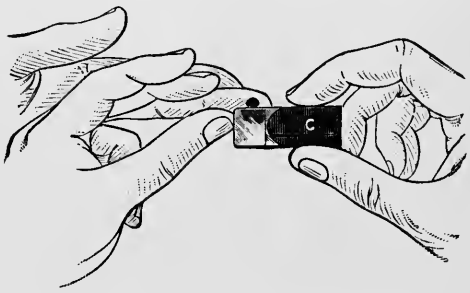


FIG. 24.—Filling the automatic blood pipette.

My personal experience with the instrument has been quite satisfactory. The readings are somewhat higher than with the Fleischl instrument.

Fleischl's Hemoglobinometer.—The principle underlying the v. Fleischl method is essentially the same as that of the Dare method; the color of the blood is compared with the color of a glass wedge stained with the golden purple of Cassius or a similar pigment, a scale indicating the corresponding amount of hemoglobin. With the Fleischl instrument, however, diluted blood is used, which is one of the disadvantages of the method.

The instrument (Fig. 25) consists of the glass wedge *a*, to which a scale, *b*, is attached, ranging from 0 to 120, 0 being placed at the thinnest, 120 at the thickest portion of the wedge. By means of a rack and pinion this may be made to slide from side to side beneath a platform corresponding to the stage of a microscope. In the centre of the platform there is a circular opening into which artificial light (daylight is not permissible) is projected from a circular plate of plaster of Paris mounted beneath, in the position of the mirror of the microscope. Into the circular opening a metallic tube, 1.5 cm. in height, is fixed, which is closed at the bottom with a plate of glass and divided into two equal compartments by a metal partition. One compartment receives the light through the glass wedge—the red chamber; the other, directly from the plaster-of-Paris reflector—the white chamber.

Capillary pipettes accompany the instrument. Their capacity is indicated on the handle of each, which number must correspond with that marked on the top screw head of the individual instrument. Generally speaking, the capacity of each pipette is such that with the blood of a perfectly normal individual the mixture of blood and water in the white chamber will correspond in color to that of the colored wedge at the mark 100 (a 13.77 per cent. solution of hemoglobin).

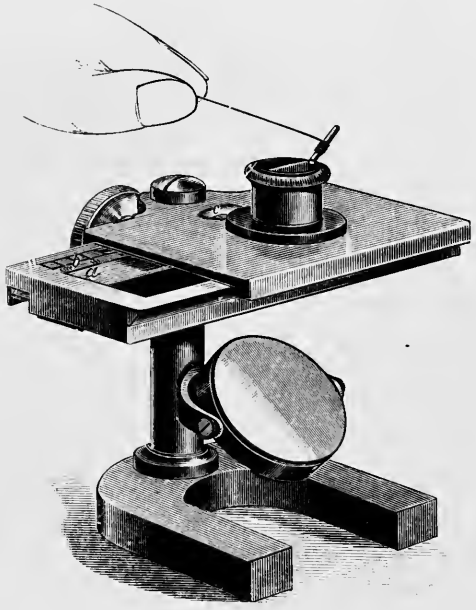


FIG. 25.— v. Fleischl's hemometer.

The pipette is filled by capillary attraction from a drop of blood obtained in the usual manner. If on trial it is found that the blood does not immediately run up in the tube, this is repeatedly washed out with water and then dried. If this is always done *after* the examination, the pipette will be in working order on the next occasion. While filling the pipette care should be had that it is not immersed in the blood, but only brought in contact with it. The two compartments of the cell having been previously partly filled with water, the charged pipette is at once placed in the white chamber and rapidly moved to and fro until the blood is well mixed with the water. Any trace remaining in the pipette is carefully washed out with water by the aid of a medicine dropper. The contents of the chamber are stirred with the handle of the pipette when both compartments are

filled with water, using the same dropper, so that there is a convex meniscus over each. The color of the blood is then matched on the wedge, which should be moved by quick turns of the adjustment screw rather than in a gradual way, as the eye will otherwise be less apt to appreciate fine shades of difference. Daylight is not permissible; a candle or gas flame of moderate intensity placed about a foot and a half distant is best. The eye should be perpendicularly above the cell, and it is well to view the colors through a paper tube, which is placed over the two compartments. The number facing the notch in the little well immediately behind the cell indicates the percentage of hemoglobin. The readings corresponding to the middle portion of the wedge are apt to be more nearly correct than the lower values. For this reason it is well, when a preliminary examination has shown a low figure, to repeat the test, using two or three pipettefuls of blood instead of one, the result, of course, being divided by 2 or 3, as the case may be. On the whole, the Fleischl method furnishes results which are somewhat lower than those obtained with the Dare; this is true especially of the older models, with which a percentage of 100 was only rarely observed. The instruments of more recent construction are much better. Personally, I regret to see the Fleischl apparatus supplanted by other instruments; it was convenient and neat. It has its defects, to be sure, and it is unfortunate that the *Miescher modification*, in which these have been eliminated, and which unquestionably gives the most accurate results, is still so costly that its general use is out of the question.

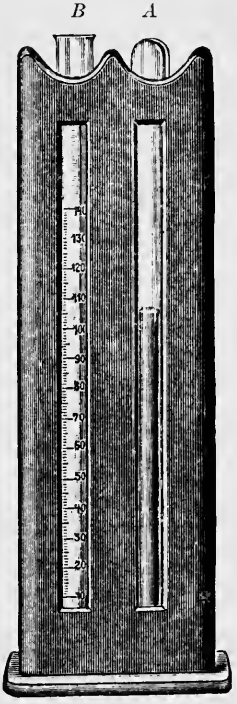


FIG. 26.—Sahli's hemoglobinometer.

Gowers' Hemoglobinometer (*Sahli's Modification*).—The apparatus (Fig. 26) consists of two glass tubes (*A* and *B*) which are of the same diameter. One of these (*A*) is closed and contains a solution of hematin hydrochlorate in a concentration corresponding to a 1 per cent. solution of normal blood. The other tube is provided with an ascending scale of 140 divisions, each degree corresponding to 20 c.mm. A capillary pipette marked at 20 c.mm., a guarded lancet, a dropping bottle, and a small stand accompany the instrument.

The finger is punctured as usual and the pipette filled to the 20 c.mm. mark; the blood is immediately discharged into the graduated tube and mixed with one-tenth normal hydrochloric acid (saturated

with chloroform as a preservative) which has been previously filled in to the mark 10. When the color of the mixture has become a clear dark brown, water is added drop by drop, shaking after every addition, until the color matches that of the standard solution. The division on the scale ultimately reached indicates the percentage of hemoglobin.

The examination can be conducted with natural and artificial light.

The method, as I have indicated above, is satisfactory if the instrument has been obtained from a reliable source. Its low cost makes it especially serviceable in large clinics and for purposes of teaching in large classes. But in every case it is advisable to compare its scale with a standard instrument. As they are marketed the individual error varies between 8 and 24 per cent., being usually on the minus side of the scale.

Talquist's Method.—The color of the blood, in this case undiluted, is compared with a series of lithographed standard tints, which represent a scale ranging by tens from 10 to 100. The technique is very simple: drops of blood are received on pieces of white filter paper of suitable thickness which accompany the color scale, and are compared with the tints on the plate, using ordinary daylight.

Accuracy is, of course, not to be expected from so crude a method, so that its use is of necessity limited. It will suffice in a very general way to control the result of treatment, but it is inapplicable in the determination of the color index.

Estimation of Blood Iron with Jolles' Ferrometer.—The estimation of the hemoglobin from the amount of blood iron, originally suggested by Jolles, is unfortunately not possible, as it has been shown that constant relations between the two bodies do not exist. All the iron of the blood is not present in this form, nor does it all occur in the form of colored compounds. Nevertheless, Jolles' method of estimating the total amount of blood iron deserves consideration, as it is a practical method and discloses facts which are of clinical interest.

The principle is the following: A small amount of blood is incinerated, and the remaining red oxide of iron brought into solution with a little monacid potassium sulphate. In this solution the iron is estimated colorimetrically with an instrument which is constructed upon the principle of Fleischl's hemometer and which is termed the ferrometer. It is made by Reichert in Vienna and can be readily transformed into a hemometer proper. Full directions accompany the apparatus. The results are expressed in relative terms, the number 100 on the scale corresponding to 0.0425 per cent. by weight of iron. Some of the results which have been obtained with the ferrometer are given below, together with the corresponding figures indicating the amount of hemoglobin:

	Ferrometer number.	Hemometer number.
Normal	103.0	100
Normal	92.6	105
Normal	95.5	100
Normal	110.0	105
Normal	83.8	92
Chlorosis	32.1 to 68.2	30 to 65
Simple anemia	33:2 to 74.7	15 to 40
Icterus	55.0	80
Leukemia	40.7	32
Leukemia	38.6	35
Pseudoleukemia	77.24	75 to 80
Severe diabetes	78.7	30
Severe diabetes	91.4	35 to 40
Parenchymatous nephritis	51.7	50

These figures at once illustrate the lack of relationship which exists between the amount of hemoglobin and that of the blood iron as a whole.

In a series of cases Jolles also examined into the presence of iron in the serum by centrifugating a given volume of blood mixed with an 0.8 per cent. salt solution, and found that in health the serum contains no iron. In 3 cases of chlorosis, in 1 case of leukemia, in 1 of neoplasm, and 1 of interstitial nephritis, negative results were likewise reached. In 2 cases of severe diabetes, on the other hand, notable quantities were found.

Deganello has studied the relation between the amount of blood iron and hemoglobin $\left(\frac{Fe}{Hb}\right)$ in different forms of secondary anemia, and found that this ratio remains normal, until the Hb has reached a certain minimum—46 to 58 per cent.; from this point off the value $\frac{Fe}{Hb}$ surpasses the normal the more the deeper the Hb value falls. Mere mechanical loss of Hb does not materially alter this value, however, even in cases of marked oligochromemia. When toxic influences are at play marked discrepancies will result.

Mitulescu comes to quite analogous conclusions. He thinks that a hemoglobin estimation only is required, as a rule, from which the iron value can be calculated according to Hoppe-Seyler's formula: $Fe = \frac{Hb \times 0.42}{100}$. If hemolytic processes are suspected, or if albuminuria exists, both estimations must be made, however, and the same is evidently necessary also in diabetes.

THE SPECIFIC GRAVITY OF THE BLOOD

The specific gravity of the blood in healthy adults varies between 1.058 and 1.062, being higher on an average in men, 1.059, than in women, 1.056, and children—boys, 1.052; girls, 1,050.

Under pathological conditions the specific gravity may vary between 1.025 and 1.083. In nephritis, chlorosis, the anemias in general, and in cachectic conditions (carcinoma of the stomach, etc.) it may diminish to 1.031. In phthisis it is diminished in the third stage (1.040 to 1.042), and in the first stage (1.049) in those patients in whom the onset has been very gradual. In the second stage normal figures are obtained (1.058 to 1.060), corresponding to the relatively high percentage of hemoglobin (90 to 95 per cent.) which is then noted, and which is referable no doubt to a concentration of the blood. An increased specific gravity is met with in febrile diseases (typhoid fever, 1.057 to 1.063), conditions associated with pronounced cyanosis (emphysema, fatty heart, uncompensated valvular disease 1.054 to 1.068), and obstructive jaundice, 1.062. The highest values have been found in enterogenous cyanosis, 1.067 to 1.083.

As the result of numerous investigations it may now be regarded as an established fact that, with the exception of nephritis, circulatory disturbances, leukemia, posthemorrhagic anemia, and anemia resulting from inanition, the specific gravity of the blood varies directly with the amount of hemoglobin and the volume of the red corpuscles. A simple method is thus given by means of which hemoglobin estimations can be made in the absence of the more expensive instruments. In the following table the specific gravities, as obtained with Hammerschlag's method, are given, with the corresponding amounts of hemoglobin:

Specific gravity according to Hammerschlag.	Hemoglobin.
1.033 to 1.035	25 to 30 per cent.
1.035 to 1.038	30 to 35 "
1.038 to 1.040	35 to 40 "
1.040 to 1.045	40 to 45 "
1.045 to 1.048	45 to 55 "
1.048 to 1.050	55 to 65 "
1.050 to 1.053	65 to 70 "
1.053 to 1.055	70 to 75 "
1.055 to 1.057	75 to 85 "
1.057 to 1.060	85 to 95 "

Method (*Hammerschlag*).—A carefully dried cylinder, measuring about 10 cm. in height, is partly filled with a mixture of chloroform (sp. gr. 1.526) and benzol (sp. gr. 0.889), having a specific gravity of 1.050 to 1.060. Into this solution a drop of blood is allowed to fall directly from the finger, pressure being avoided, and care taken that the drop does not come in contact with the walls of the vessel. The drop should not be too large, as otherwise it will separate into droplets, giving rise to inaccurate results. Should the drop sink to the bottom, it is apparent that the specific gravity of the mixture is lower than that of the blood, necessitating the addition of chloroform. This should be added drop by drop while the mixture is thoroughly stirred. If, on the other hand, the drop should tend

toward the surface it is best to add an amount of benzol sufficient to cause the blood to sink to the bottom, and then to bring it to the proper degree of suspension by the subsequent addition of chloroform. As soon as the drop remains suspended the mixture is filtered, and its specific gravity ascertained by means of an accurate hydrometer registered to the fourth decimal. The figure obtained is the specific gravity of the blood. The chloroform-benzol mixture may be kept indefinitely.

Instead of the chloroform-benzol mixture, one of chloroform and olive oil may be employed, as suggested by Van Spanje. It has the advantage of being less volatile than the other. Three parts of chloroform and one of oil give a mixture with a specific gravity of 1.056.

THE REACTION OF THE BLOOD

Viewed from a physico-chemical standpoint the reaction of the blood is practically neutral. Chemically speaking, however, it is alkaline, owing to the presence of disodium phosphate and sodium carbonate. The degree of alkalinity in healthy adults, while fasting, corresponds to about 300 to 325 mgs. of sodium hydrate for 100 c.c. of blood (Löwy). Variations amounting to 75 mgs. plus or minus are, however, not uncommon and in part due to unavoidable errors of technique (30 mgs.).

Generally the alkalinity of the blood is lower in women and children than in men, and is influenced by the process of digestion, exercise, etc. At the beginning of digestion, when hydrochloric acid is being freely secreted, the alkalinity of the blood increases; while later on it diminishes. Higher values are usually found during pregnancy than in the non-pregnant state. A decrease is observed following violent muscular exercise and also after the prolonged use of acids, while an increase is brought about by the ingestion of alkalies. An increase in the alkalinity of the blood occurs after a cold bath, and it is interesting to note that this is apparently associated with an increase in the bactericidal power of the blood.

Under pathological conditions the alkalinity may be diminished or increased, as is shown in the table below. Unfortunately we are not able to account for these fluctuations in a satisfactory manner, and the data are thus of little value. A marked decrease in diabetes may be viewed as a serious omen and as indicating marked acid intoxication. During diabetic coma the reaction, owing to the presence of large amounts of β-oxybutyric acid, may actually be acid. The supposition that in gout a diminished alkalinity exists in the intervals between attacks, and that this increases beyond the normal during the attack, has been proved unfounded.

Orlowsky has expressed the opinion that the variations in the alkalinity of the blood which have been noted in various diseases and

sometimes in one and the same disease, by various investigators working with the older methods, are referable to the varying tonicity of the blood and its varying richness in red corpuscles. Working with blood plasma, he found a marked diminution of the alkalinity in advanced uremia, in cancerous cachexia, and in severe cases of diabetes, while in other diseases normal values or at most but slight and exceptional variations were observed.

The following table gives some of the results which have been obtained with Dare's method. The results are expressed in terms of the number of c.c. of the tartaric acid solution employed to bring about the end reaction (see below). The corresponding values of sodium hydrate are seen below.

	Average c.c. of tartaric acid solution.	Color index.
Normal	1.6	0.95 to 1.17
Typhoid fever (22 estimations)	0.8 to 2.3	0.64 to 1.3
Myelogenous leukemia (1 case)	0.8	0.65
Splenic anemia (1 case)	1.8	0.81
Catarrhal jaundice (2 cases)	1.6 to 2.1	0.74 to 0.75
Liver abscess (1 case)	0.3	0.58
Croupous pneumonia (2 cases)	1.5	0.71
Pulmonary tuberculosis (1 case)	1.55	0.70
Meningeal tuberculosis (1 case)	2.35	0.98
Peritoneal tuberculosis (2 cases)	0.4 to 1.6	0.32 to 0.67
Glandular tuberculosis (3 cases)	1.05 to 1.1	0.59 to 0.85
Gastric ulcer (3 cases)	0.9 to 1.45	0.68 to 0.88
Malignant disease (8 cases)	1.1 to 2.0	0.46 to 0.97

Dare's Method.—This method is based upon the fact that the characteristic spectrum of oxyhemoglobin disappears at the point of exact neutralization when the blood is titrated with a dilute solution of tartaric acid.

The examination is made with the aid of a special instrument, the *hemo-alkalimeter*, which is pictured in the accompanying illustration (Fig. 27). *B* is a glass stopper through which passes an automatic capillary blood pipette of 20 c.mm. capacity, the exposed end of which is ground to a tapering point. The stopper fits into the tube *A*, which has a capacity of 3 c.c. and is graduated in cubic centimeters. The upper end of the tube is blown into a bulb with a minute aperture at *C*. A 2 c.c. dropping tube provided with a short piece of rubber tubing accompanies the instrument.

To neutralize the blood a $\frac{1}{200}$ normal solution of tartaric acid is used, which should contain an amount of alcohol sufficient to prevent the growth of bacteria, but insufficient to precipitate the albumins of the blood. The reagent may be prepared by dissolving 0.075 gram of tartaric acid (Merck's crystals; guaranteed reagent) in a small amount of distilled water, adding 20 c.c. of alcohol (93 to 94 per cent.), and diluting to 200 c.c. with water.

For the spectroscopic examination a Browning instrument (Fig. 28) will suffice.

Technique.—A drop of blood is obtained from the finger-tip or the lobe of the ear in the usual manner. The blood pipette is filled *in situ* by capillary attraction, holding the instrument horizontally to the drop of blood as it emerges from the wound. With an ordinary medicine dropper filled with distilled water the blood is washed into the bottom of the tube, connecting the dropper with the pipette by means of a short piece of rubber tubing. Blood and water should just reach the zero mark, and are intimately mixed by closing the aperture in the bulb with the finger and inverting the tube several times. The *reagent pipette* is then filled with the tartaric acid solution and the rubber tubing slipped over the outer end of the blood pipette; by compressing the rubber bulb the acid solution is forced through the pipette into the test-tube, the aperture in the glass bulb being closed before the pressure is relaxed. Having done this, the tube is inverted several times while still attached to the reagent

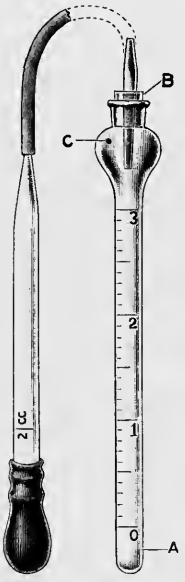


FIG. 27.—Dare's hemo-alkalimeter.

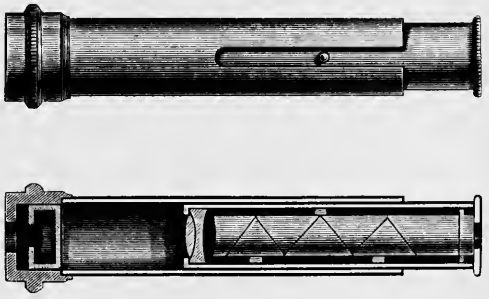


FIG. 28.—Browning's spectroscope. (Zeiss.)

pipette, taking care that this is held vertically so that the acid solution does not get into the rubber bulb. The tube is clamped in front of the spectroscope and examined for the two bands of oxyhemoglobin. So long as these are visible more of the acid is added, inverting the tube after each addition; as the bands become fainter one drop at a time is allowed to enter. At first this is rather tedious, but after several examinations have been made it will be found unnecessary to apply the spectroscope so frequently to determine the point of neutralization, as the eye rapidly learns to recognize this by the characteristic change of color of the blood mixture. The observation is at an end when the oxyhemoglobin bands have just disappeared.

The examination is made with artificial light, keeping the distance from the light constant.

Dare suggests that for sake of convenience the results be expressed in terms of the number of cubic centimeters of the tartaric acid

solution instead of in milligrams of sodium hydrate, as has been customary. The corresponding values are given in the table below, and have reference to 100 c.c. of blood. His average normal value is 1.6 = 212 mg. of NaOH for 100 c.c. of blood.

C.c. of reagent.	Equivalent in terms of mg of NaOH per 100 c.c. of blood.
2.6	345.0
2.4	319.0
2.2	292.0
2.0	266.0
1.8	239.0
1.6	212.0
1.4	176.0
1.2	169.0
1.0	133.0
0.8	96.0
0.6	79.0
0.4	53.0
0.2	26.6

Dare has ascertained with his method that there is a more or less constant relationship between the alkalinity of the blood and the color index (see table above), and he suggests that this may be the reason why the results obtained by different investigators differ so widely, as *at different stages of the disease the color index may change.*

The method is quite convenient and merits the attention of all laboratory workers.

THE DETERMINATION OF THE EXISTENCE OF ACIDOSIS.

Determination of the Bicarbonate Content of the Blood.—Since the determination of the degree of acidosis, by urinary examination can have reference only to the *excretion* of the acid bodies in question and not to the extent of their *formation,* unless the two processes run a parallel course, it has been suggested that a better insight into the extent of acid intoxication might be gained by a determination of the bicarbonate content of the blood. This stands to reason, but was not practical in the past as no simple method was extant which could be applied in the clinical laboratory. Since the introduction of Van Slyke's method, however, such determinations are now perfectly practical and should be freely made in suitable cases.

Van Slyke's Method.—From 5 to 10 c.c. of blood are drawn from a vein into a test tube containing a sufficient quantity of potassium oxalate powder to make its content 1 per cent. or a little less. The tube is centrifugalized and the plasma pipetted off. Slight hemolysis does not appreciably affect the results, but it is advisable to centrifugalize the blood as soon as possible. After centrifugation, the plasma may be kept in a paraffin-lined test tube, on ice, for as long as a week, without deterioration.

In order to determine the alkaline reserve of the specimen, the plasma is saturated with carbon dioxide at alveolar tension. To this end, about 3 c.c. of the plasma (at room temperature) are placed in a separating funnel of about 300 c.c. capacity, and the funnel filled with alveolar air from the lungs of the operator, the air being passed through a bottle filled with glass beads, before it enters the funnel, so as to bring the moisture content down to saturation at room temperature. The funnel is closed just before the stream of breath stops, and the plasma shaken for one minute in such a manner as to bring it in as extensive a contact with the walls of the funnel as possible. In this way equilibrium with the carbon dioxide content of the air is quickly approached. This process is repeated several times, when the determination of the carbon dioxide content of the saturated plasma is performed as follows, using the carbon dioxide apparatus, pictured in the accompanying illustration (Fig. 29).[1] The apparatus, firmly *held in a strong clamp,* is completely filled with mercury, including the two capillaries above the upper stopcock. The mercury levelling bulb is placed almost on a level with the lower cock. The cup at the top of the apparatus is washed out well with dilute ammonia, followed by water (using medicine droppers for the purpose). One c.c. of the saturated plasma is placed in the cup and allowed to flow down into the upper stem of the instrument. The cup is now washed with two portions of about 0.5 c.c. each of water, the washings being allowed to flow into the stem containing the plasma, care being taken, however, that no air enters the instrument with the liquid. One small drop of octyl alcohol (to prevent foaming) is placed in the capillary connecting the cup with the upper end of the apparatus, and about 1 c.c. of 5 per cent. sulphuric acid is poured into the cup. Enough of the latter is now admitted into

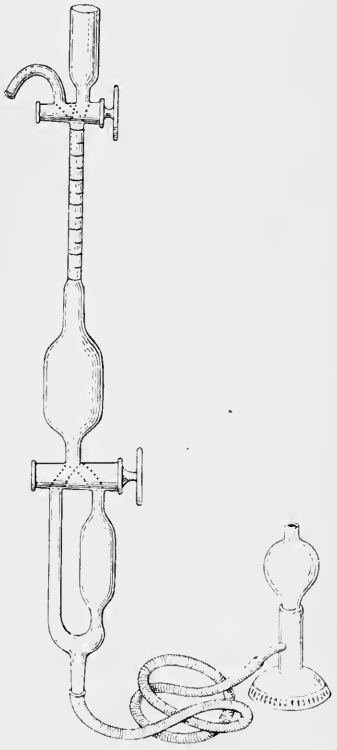


Fig. 29.—Van Slyke's apparatus for measuring the carbon dioxide content of the blood.

[1] The apparatus may be obtained from E. Greiner, 55 Fulton Street, New York.

the apparatus, carrying the octyl alcohol with it, so that the total volume of fluid in the apparatus is exactly 2.5 c.c. A drop of mercury is now placed in the cup and allowed to flow to the upper stopcock in order to seal this and make it capable of holding an absolute vacuum. The levelling bulb (the lower cock having remained open from the beginning of operations) is now lowered to a level where the surface of the mercury in it is about 300 mm. below the lower stopcock, and the mercury in the apparatus allowed to fall until the meniscus of the mercury has dropped to the 50 c.c. mark of the apparatus. While this is being done it will be noted that bubbles of carbon dioxide escape from the fluid into the vacuum. In order to bring about the complete and rapid removal of all the carbon dioxide the apparatus is removed from its clamps and turned upside down about a dozen times. It is then reclamped (the levelling bulb still being at its low level) and the fluid allowed to flow through the lower stopcock into the small bulb below. This removal should be as complete as possible, care being taken, however, that no gas escapes from above the lower stopcock. The levelling bulb is raised in the left hand, and the lower stopcock turned with the right, so that mercury is admitted through the left-hand entrance of the three-way cock without readmitting the plasma mixture. The levelling bulb is now brought to a position where the mercury in the bulb and the apparatus are on the same level, and the gas in the latter hence under atmospheric pressure. A few hundredths of a c.c. of water will float on the mercury, but this may be disregarded in levelling. The volume of gas is then read off at once. The corresponding number of c.c. of carbon dioxide chemically bound by 100 c.c. of plasma will be found in the table below. The temperature figures at the head of the columns represent the room temperatures at which the samples of plasma are saturated with carbon dioxide and analyzed, it being assumed that both operations are performed at the same temperature. If the figures in the table are multiplied by 0.94, the volume percentage of carbon dioxide bound by the plasma at body temperature (37° C.), is obtained (within 1 or 2 per cent.), and if the figures in the table are multiplied by 0.69, they give, usually within less than 5 mm. the alveolar carbon dioxide tension of the plasma donor, as determined by the Fredericia apparatus.

If the figures in the table are multiplied by 0.01964, viz., the weight of 1 c.c. CO_2 at 0° C. and 760 Hgm., they give in mgrms. the carbon dioxide bound by 1 c.c. of plasma.

With this method Van Slyke found that the plasma of normal adults yields 0.65 to 0.90 c.c. of gas, corresponding to 55 to 77 volume per cent. of carbon dioxide, chemically bound by the plasma. Figures lower than 50 per cent. in adults are found to indicate acidosis, which in infants 40 to 50 per cent. appears to be normal.

Observed vol. gas B X 760	Cc. of CO_2 chemically bound by 100 c.c. plasma. 15°	20°	25°	30°	Observed vol. gas B X 760	Cc. of CO_2 chemically bound by 100 c.c. plasma. 15°	20°	25°	30°
0.20	9.1	9.9	10.7	11.8	0.60	47.7	48.1	48.5	48.6
1	10.1	10.9	11.7	12.6	1	48.7	49.0	49.4	49.5
2	11.0	11.8	12.6	13.5	2	49.7	50.0	50.4	50.4
3	12.0	12.8	13.6	14.3	3	50.7	51.0	51.3	51.4
4	13.0	13.7	14.5	15.2	4	51.6	51.9	52.2	52.3
5	13.9	14.7	15.5	16.1	5	52.6	52.8	53.2	53.2
6	14.9	15.7	16.4	17.0	6	53.6	53.8	54.1	54.1
7	15.9	16.6	17.4	18.0	7	54.5	54.8	55.1	55.1
8	16.8	17.6	18.3	18.9	8	55.5	55.7	56.0	56.0
9	17.8	18.5	19.2	19.8	9	56.5	56.7	57.0	56.9
0.30	18.8	19.5	20.2	20.8	0.70	57.4	57.6	57.9	57.9
1	19.7	20.4	21.1	21.7	1	58.4	58.6	58.9	58.8
2	20.7	21.4	22.1	22.6	2	59.4	59.5	59.8	59.7
3	21.7	22.3	23.0	23.5	3	60.3	60.5	60.7	60.6
4	22.6	23.3	24.0	24.5	4	61.3	61.4	61.7	61.6
5	23.6	24.2	24.9	25.4	5	62.3	62.4	62.6	62.5
6	24.6	25.2	25.8	26.3	6	63.2	63.3	63.6	63.4
7	25.5	26.2	26.8	27.3	7	64.2	64.3	64.5	64.3
8	26.5	27.1	27.7	28.2	8	65.2	65.3	65.5	65.3
9	27.5	28.1	28.7	29.1	9	66.1	66.2	66.4	66.2
0.40	28.4	29.0	29.6	30.0	0.80	67.1	67.2	67.3	67.1
1	29.4	30.0	30.5	31.0	1	68.1	68.1	68.3	68.0
2	30.3	30.9	31.5	31.9	2	69.0	69.1	69.2	69.0
3	31.3	31.9	32.4	32.8	3	70.0	70.0	70.2	69.9
4	32.3	32.8	33.4	33.8	4	71.0	71.0	71.1	70.8
5	33.2	33.8	34.3	34.7	5	71.9	72.0	72.1	71.8
6	34.2	34.7	35.3	35.6	6	72.9	72.9	73.0	72.7
7	35.2	35.7	36.2	36.5	7	73.9	73.9	74.0	73.6
8	36.1	36.6	37.2	37.4	8	74.8	74.8	74.9	74.5
9	37.1	37.6	38.1	38.4	9	75.8	75.8	75.8	75.4
0.50	38.1	38.5	39.0	39.3	0.90	76.8	76.7	76.8	76.4
1	39.1	39.5	40.0	40.3	1	77.8	77.7	77.7	77.3
2	40.0	40.4	40.9	41.2	2	78.7	78.6	78.7	78.2
3	41.0	41.4	41.9	42.1	3	79.7	79.6	79.6	79.2
4	42.0	42.4	42.8	43.0	4	80.7	80.5	80.6	80.1
5	42.9	43.3	43.8	43.9	5	81.6	81.5	81.5	81.0
6	43.9	44.3	44.7	44.9	6	82.5	82.5	82.4	82.0
7	44.9	45.3	45.7	45.8	7	83.6	83.4	83.4	82.9
8	45.8	46.2	46.6	46.7	8	84.5	84.4	84.3	83.8
9	46.8	47.1	47.5	47.6	9	85.5	85.3	85.2	84.8
0.60	47.7	48.1	48.5	48.6	1.00	86.5	86.2	86.2	85.7

Marriott's Method.—Excellent results may also be obtained by means of Marriott's method. In this case a certain amount of serum or whole blood is dialyzed against salt solution and the hydrogen ion concentration of the dialysate determined colorimetrically with acid-basic phosphate mixtures of known ion concentration, using phenolsulphonephthalein as an indicator.

REAGENTS.—1. *The Standard Acid-alkaline Phosphate Mixtures.*—These are prepared from $\frac{N}{15}$ acid and basic phosphate solutions (see p. 103), in the proportions indicated below, which correspond to the hydrogen ion concentrations noted in the top line.

PH[1]	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.6
Acid phosphate sol. c.c.	37.0	27.0	19.0	13.2	8.8	5.6	3.2	2.0	1.0
Basic phosphate sol. c.c.	63.0	73.0	81.0	86.8	91.2	94.4	96.8	98.0	99.0

Test tubes are charged with the mixtures, as described on p. 103, and if protected against the light retain their color shades for a long period of time.

[1] Indicating the negative logarithm of the hydrogen ion concentration.

2. *Dialyzing Tubes.*—These may be conveniently prepared in the laboratory from a solution of celloidin, as follows:

One ounce of celloidin (Anthony's negative cotton) is dissolved in 500 c.c. of a mixture of equal parts of ether and 95 per cent. alcohol. After standing for a week to allow impurities to settle, the dialyzing tubes are prepared by pouring a small amount of the solution into a little test tube (measuring about 50 by 6 mm., with a flaring mouth) and slowly pouring it out again, while rotating the tube. In this manner the interior of the tube receives a fine coating of celloidin. The tube is held upside down until the odor of ether has disappeared. It is then filled with cold water, the celloidin loosened from the edge with the blade of a knife, and water allowed to flow into the resulting space between the tube and the sack, when the latter can easily be dislodged, and may now be kept until needed, immersed in a jar of water. Such dialyzers remain serviceable for a long time, but should be tested from time to time in regard to their permeability to proteins, on the one hand, and salts on the other.

3. *The Salt Solution.*—This should contain 8 gm. of chemically pure sodium chloride, plus 220 c.c. of a 0.01 per cent. solution of phenolsulphonephthalein, per liter of distilled water. The glass receptacle must be free from alkali (Jena or non-sol.), and any carbon dioxide present expelled by a current of air that has passed through a strong solution of sodium hydrate. The resultant solution after cooling is compared with the phosphate standards and should correspond to 7; if necessary it is brought to this point by the addition of a few drops of very dilute acid or alkali to the whole solution.

Method.—A few c.c. of blood are secured from a vein in the usual manner, and the serum separated from the corpuscles by centrifugation as soon after withdrawal from the patient as possible. 0.5 c.c. of the serum is placed in one of the celloidin dialyzers, after this has been rinsed inside and out in saline, and the thimble placed in a test tube, measuring about 50 by 8 mm., and containing 2 c.c. of the indicator salt solution. The level of the latter should be at least as high as that of the serum in the thimble. After seven minutes the latter is removed, the dialysate placed in a clean test tube of the same diameter as those containing the standard phosphate solutions (100 to 140 mm. in length), when a rapid current of air is allowed to bubble through the solution (to remove carbon dioxide). This takes about three minutes, after which the color of the solution is compared with the standard, interpolating if necessary (see p. 104). The resultant figure indicates the reserve alkalinity of the blood in terms of the negative logarithm of its hydrogen ion concentration. This is designated by the symbol RpH.

Results.—Under normal conditions Marriott obtained values ranging between 8.4 and 8.55, the individuals being on an ordinary mixed diet (not fasting). This corresponds to a carbon dioxide tension of

from 38 to 45 mm. Values between 8.0 and 8.3 (tension of from 28 to 35) were found to be indicative of a moderate degree of acidosis. A value as low as 7.7 (tension of 20 mm.) pointed to imminent danger. During coma a value as low as 7.3 (11 mm.) was observed.

In infants under one year the normal values may be a little lower than in the adult, viz., 8.3 (35 mm.).

Marriott further states that unless the RpH of the serum is below 7.9 the acidosis may be successfully combated by dietetic regulation or by the administration of alkali by mouth. When the RpH of the serum falls below 7.9 intravenous administration of alkali is usually indicated.

The Determination of the Alveolar Carbon Dioxide Tension.—Another very satisfactory method for estimating the degree of acidosis has been devised by Marriott and is based on the determination of the carbon dioxide tension of the alveolar air.

Marriott's Method. — *Principle.* — The patient is allowed to rebreathe the air contained in a suitable receptacle, for a certain length of time, when the resultant carbon dioxide is passed through a solution of sodium carbonate or bicarbonate to the point of saturation. The final solution will then contain sodium bicarbonate and carbon dioxide. The resultant reaction depends upon the ratio of the carbon dioxide to the alkaline bicarbonate present, and this in turn upon the tension of the carbon dioxide in the sample of air undergoing analysis. The volume of air passed through the solution is immaterial providing that saturation with carbon dioxide be attained. As a high tension of the gas will turn the reaction of the solution toward the acid, and a low tension toward the alkaline side, the resultant reaction is an index of the degree of the carbon dioxide tension in the alveolar air. The reaction of the solution Marriott estimates colorimetrically by comparing the color of the resultant solution, after the addition of phenolsulphonephthalein, with that of corresponding mixtures containing known amounts of acid and alkaline phosphates.

Preparation of the Solutions.—1. *The Standard Bicarbonate Solution.*—This contains 0.53 gm. of desiccated sodium carbonate plus 200 c.c. of a 0.01 per cent. solution of phenolsulphonephthalein to the liter. Or, in its place 100 c.c. $\frac{N}{10}$ sodium hydrate solution plus the same volume of the phthalein solution may be used, the volume being brought to the 1000 c.c. mark with distilled water. In either case bicarbonate will be formed when the alveolar air is passed through the solution.

2. *The Standard Acid-alkaline Phosphate Mixtures.*—A $\frac{N}{15}$ acid potassium phosphate solution is prepared by dissolving 9.078 gm. of the pure, recrystallized salt in 200 c.c. of a 0.01 per cent. solution of phenolsulphonephthalein solution and diluting to 100 c.c.

In addition, a $\frac{N}{15}$ solution of disodium phosphate is prepared. To this end a quantity of the pure recrystallized salt of the composi-

tion $Na_2HPO_4.12H_2O$ is exposed to the air, protected from dust, for about two weeks. During this time the salt loses ten molecules of water, and a product results, having the composition $Na_2HPO_4.2H_2O$. Of this 11.876 gm. are dissolved in 200 c.c. of a 0.01 per cent. solution of phenolsulphonephthalein, and the volume brought to the 100 c.c. mark.

The following mixtures are then prepared, corresponding to carbon dioxide tensions, as indicated:

	10	15	20	25	30	35	40	45
Acid phosphate sol. c.c.	17.8	25.2	31.0	35.7	40.5	45.0	47.0	50.2
Alkaline phosphate sol. c.c.	82.2	74.8	69.0	64.3	59.5	55.0	53.0	49.8

Specimens of these standard colored solutions are put up in small Jena or non-sol. test tubes (10 x 75 mm.), which are corked or sealed, preserved with a little toluol or thymol, and kept in the dark when not in use. The colorimetric estimations are made in a small stand, like that used with the Sahli hemoglobinometer, but provided with three slits instead of two.

Collection of the Alveolar Air.—A rubber bag of about 1500 c.c. capacity is connected by a rubber tube with a glass mouth-piece (about half an inch in diameter). By means of an atomizer bulb the bag is a little less than half-filled (about 600 c.c.) with air, and the rubber tube clamped off. The subject under observation, while in a recumbent posture, and at the end of an *expiration*, takes the tube into his mouth, an attendant holds his nose closed, and the individual now breathes back and forth into the bag four times in twenty seconds, the attendant marking the time. The tube is then immediately clamped off and the analysis started within three minutes, as carbon dioxide rapidly escapes through rubber.

For young children or comatose patients a suitable mask is required, which may either be improvised, or purchased from the dealers.[1]

Analysis.—A test tube of the same diameter as those holding the standard acid-basic phosphate solution, but measuring from 100 to 150 mm. in length, is charged with 2 or 3 c.c. of the standard bicarbonate solution and air from the bag forced through the reagent (by means of a finely drawn-out glass tube), until no further change in color takes place, viz., until the solution is saturated with carbon dioxide. It is recommended that the operator first blow his breath through the solution, so as to bring it into approximate equilibrium with alveolar air, when saturation may be effected with as little as 100 c.c. of air from the bag. The tube is then stoppered, and the color compared with the standard solutions. The color standard having been prepared for a temperature between 68° and 77° F., it is suggested to immerse the specimens in water of corresponding temperature, if the room temperature should be lower or

[1] Messrs. Hynson & Westcott, Charles and Franklin Streets, Baltimore, Md.

higher. The differences, however, are practically negligible. Duplicate determinations should of course be made in every instance, and should not differ by more than 2 mm.

Results.—Under normal conditions the tension in adults, at rest, varies from 40 to 45 mm. Tensions between 30 and 35 are found in acidosis of mild degree, while a value of 20 mm. may be regarded as indicating imminent danger. In coma the tension may be as low as 8 to 10 mm.

In infants the tension is from 3 to 5 mm. lower than in adults. While changes in the excitability of the respiratory centre, referable to factors other than acidosis, have a certain effect upon the alveolar tension, this is rarely sufficient to be of clinical significance (*e. g.*, caffein, intracranial lesions, morphin, certain infections).

THE COAGULATION OF THE BLOOD

Under normal conditions blood clots in from two to six minutes after being shed, while in disease, coagulation may be greatly retarded. This is notably seen in hemophilia, when coagulation may be delayed for hours, or may not occur at all. Lesser grades of delayed coagulation may be observed in scurvy, jaundice, typhoid fever, phosphorus poisoning, nephritis, in various forms of anemia and hepatic disease. Delayed coagulation is also quite common in patients with goitre, irrespective of the functional activity of the gland. The same is seen quite frequently in various affections of the other glands of internal secretion, as well as in general neuropathy and in the status hypoplasticus. Associated with the delayed coagulability in disorders involving the endocrine glands is a lymphocytosis, or mononucleosis and in some an eosinophilia. Howell finds that while in hemophilia the delayed coagulation is due to a deficiency in prothrombin, in other hemorrhagic conditions neither prothrombin nor antithrombin shows any deviation from the normal amount. In cases of thrombosis he found the antithrombin diminished.

In purpura, in contradistinction to hemophilia, there is almost normal clotting time, while the bleeding time is increased.

In order to estimate the rapidity of coagulation, the following method may be employed:

Howell's Method.—This is probably the most exact method of estimating the coagulation time of the blood and destined to replace those which have been in common use up to the present time.

Four c.c. of blood are obtained from a vein at the bend of the elbow in the usual manner, and immediately mixed with 0.5 c.c. of a 1 per cent. solution of sodium oxalate in 0.9 per cent. saline solution. The corpuscles are thrown down by centrifugation and the plasma recalcified with a 0.5 per cent. solution of calcium chloride. To this end a series of small tubes are charged with five

drops of plasma and one, two, and three drops respectively of the calcium chloride solution. The time is noted when the latter is added and again when coagulation has occurred, that tube being selected as representative in which *complete* coagulation first takes place. This usually occurs in tube 2 or 3, in from nine to twelve minutes.

As the result of a study with this method of over a hundred cases, both normal and pathological, Howell concludes that delayed coagulation occurs only in true hemophilia. In such cases the coagulation time varied between 90 and 300 minutes. Purpuric blood showed a normal clotting time.[1]

Method of Lee and White.—Lee and White withdraw 1 c.c. of blood from an arm vein, using a small (20 minim.) hypodermic syringe with a platinum needle. The needle is previously sterilized and rinsed out with normal salt solution. The time of withdrawal is accurately noted and the syringe immediately emptied into a small glass tube (about 8 mm. in diameter) which has likewise been previously rinsed in saline. The tube is rotated endwise every thirty seconds and the time noted when the blood no longer flows, but maintains its position. This is the end point.

Working with this method the writers found a normal coagulation time of five to eight minutes, as compared with one of two to four minutes when blood from the ear was used. Accelerated coagulation (three and one-half minutes or less) was noted in five tyhpoid cases, four of which subsequently developed thrombosis, as also in isolated cases of myelogenous leukemia, diabetes, typhus fever, and endocarditis.

Delayed coagulation was observed in 47 of 125 cases examined, viz., in 2 cases of hemophilia (almost an hour), 1 of purpura (ten to fifteen minutes), one each of splenic anemia, acute lymphatic leukemia, and myelogenous leukemia (ten to fifteen minutes), several cases of pernicious anemia (ten to fifteen minutes), various cases of jaundice (the delay was here noted especially when the jaundice was marked, while slight cases showed no deviations from the normal), 6 cases of pneumonia (six and one-half to thirty-five minutes), 7 of 16 cases of typhoid fever, 2 cases of staphylococcus and streptococcus infections, etc. The writers emphasize the prognostic value of these examinations and illustrate this by an account of the clinical findings.

THE BLOOD PIGMENTS

Hemoglobin and Oxyhemoglobin.—On spectroscopic examination hemoglobin in suitable dilution shows a single band of absorp-

[1] The above data are based upon a personal communication from Dr. Howell, and do not comprise cases of icterus with a tendency to bleeding, no opportunity having as yet occurred to study such instances.

tion between D and E, extending slightly beyond D to the left (Fig. 30).

Oxyhemoglobin shows two bands of absorption between D and E. One band, a, which is not so wide as the second, B, but darker and more sharply defined, borders on D; the second, which is wider but less sharply defined, lies at E (Fig. 31). This spectrum can be readily transformed into that of hemoglobin by the addition of a reducing agent, such as ammoniacal solution of ferrous tartrate (Stokes' fluid), ammonium sulphide, or cuprous salts.

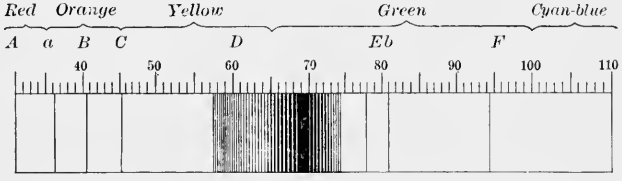


FIG. 30.—Spectrum of reduced hemoglobin. (v. Jaksch.)

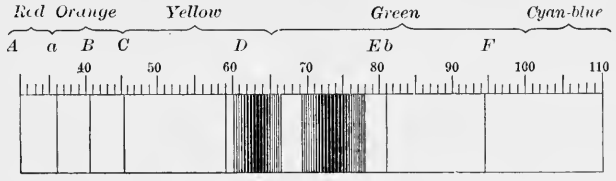


FIG. 31.—Spectrum of oxyhemoglobin. (v. Jaksch.)

Under normal conditions the amount of hemoglobin is fairly constant, but varies somewhat in different countries with the habits of the people, the character of the diet, etc. In Germany, as the result of 61 estimations, Leichtenstern found 14.16 per cent. by weight as the average in healthy men, and 13.10 per cent. in women.

Clinically we express the amount of hemoglobin by relative figures as compared with the average normal percentage by weight; on this basis the scale of the various hemoglobinometers is constructed. On these instruments the figure 100 represents the average normal value; this, however, varies somewhat with the various forms of hemoglobinometers according to the average percentage by weight which has been taken as a standard in establishing the 100 mark. With the Gowers instrument, Strauss and Rohnstein obtained figures varying between 85 and 125 as normal values which would furnish an average of 105. Schaumann and v. Willebrandt give 88 as the average normal. With the v. Fleischl instrument I have rarely found higher values than 90 per cent., but with the Dare apparatus the average results more nearly approach the 100 mark. (See Estimation of Hemoglobin.)

In children the average values are somewhat lower than in the adult. Stierlin gives 79.7 per cent. for boys and 82.1 for girls. Borchmann's values are even lower, viz., 55 and 80; Gundobin gives 70 and 95.

The ingestion of large amounts of water does not cause a dilution of the blood and hence a diminution of the amount of hemoglobin; but relatively higher values are found upon the withdrawal of liquids, owing to a concentration of the blood as a whole. Fat persons show smaller values than correspond to their age.

An increased amount of hemoglobin is termed *hyperchromemia*, while a decrease is spoken of as *oligochromemia* or *hypochromemia*.

Oligochromemia is very common, while hyperchromemia is rare. This at least is true of an *absolute* increase in the hemoglobin content of the body as a whole, while a *relative* increase, just as a relative polycythemia, is common. The same considerations which have already been discussed in connection with the latter condition also hold good for relative oligochromemia. Absolute hyperchromemia is seen almost exclusively in chronic enterogenous cyanosis and in connection with certain types of congenital heart disease (which see). A decrease in the amount of hemoglobin is, generally speaking, more frequent than oligocythemia and more extensive in its degree. In chlorosis and splenic anemia more particularly the existing anemia is essentially due to a diminished content of hemoglobin and to a less degree to a loss of red corpuscles. The most extreme grade of oligochromemia is seen in the diseases just mentioned, in malarial intoxication, in infections with streptococci and staphylococci, especially in those of puerperal origin, in certain cases of malignant disease, in the later stages of leukemia, and in pernicious anemia, though the corpuscular decrease in the last disease almost invariably exceeds the loss of hemoglobin (see color index). In some of the conditions mentioned the hemoglobin value may drop to 20 or even lower; in some cases the blood is almost devoid of color.

Hemoglobinemia.—The term hemoglobinemia has been applied to a condition in which the hemoglobin is dissolved out from the red corpuscles, and, appearing in the plasma as such, leads at first to a very decided choluria and in extreme cases to hemoglobinuria.

Various poisons, such as potassium chlorate, carbolic acid, pyrogallic acid, naphthol, arsenic, antimony, hydrochloric acid, sulphuric acid, antifebrin, antipyrin, phenacetin, sulphonal, tincture of iodine, when given hypodermically, or even internally in sufficiently large doses, will call forth a hemoglobinemia which is followed by hemoglobinuria.

Fresh morels also contain a poison which is capable of producing an intense hemoglobinuria, and which may be extracted with hot water.

In acute and chronic infectious diseases of a severe type, such as

scarlatina, typhoid fever, intermittent fever, icterus gravis, syphilis, as also in diseases depending upon a hemorrhagic diathesis, such as variola hemorrhagica, scurvy, as also following insolation, extensive burns, and frostbite, hemoglobinemia, leading to hemoglobinuria, is not infrequently observed. The same has been noted in splenic anemia and in Raynaud's disease. In syphilis a moderate grade of hemoglobinemia can be demonstrated by spectroscopic examination of the serum within two or three minutes following an intravenous injection of mercuric chloride in medicinal doses. (See also Justus' Test.)

An epidemic hemoglobinuria of the newly born and a paroxysmal or intermittent hemoglobinuria, both of unknown origin, have likewise been described.

Hemoglobinemia also follows the infusion of blood of animals of one species into the circulation of animals of a different species, and, as is now well known, the serum of certain humans also is hemolytic for the red cells of others. This is important to bear in mind in transfusion work, and it is now the rule to test the sera of donors and recipient against each others' corpuscles before such an operation unless there are urgent reasons to the contrary. To this end one may proceed as follows:

Transfusion Test of the Author.—Two specimens of blood are secured from the recipient and each donor, the one set to furnish the sera (S), the other the corpuscles (C). To procure the former, half a dozen drops of blood are collected from the ear in a small glass tube from each donor and double that number from the recipient. The specimens are labeled Rs, D Is, D IIs, D IIIs, D IVs and so on. On clotting, the clot is loosened from the walls of the tubes and the serum separated by centrifugation.

To procure the corpuscles two or three drops of blood are similarly obtained from each person, but allowed to flow directly into little tubes, each containing approximately 5 c.c. of a 1 per cent. solution of sodium oxalate in 0.9 per cent. saline. These tubes are correspondingly labeled Rc, D Ic, D IIc, D IIIc, D IVc, etc. The corpuscles are thrown down by centrifugation, the supernatant fluid pipetted off, and the corpuscles again brought into suspension in a corresponding quantity of 0.9 per cent. saline. This should be as homogeneous as possible, and if necessary clumps may be thrown down by centrifugalizing for a few moments.

A series of slides, the number corresponding to the number of donors, is now prepared, each with two little squares lined by a little wall of vaseline. The left squares receive a drop of the recipient's serum and a drop of the donor's corpuscle emulsion, while the right squares are charged with a drop of the recipient's corpuscle emulsion and a drop of the donor's serum.

The specimens are then incubated for one-half hour at 37° C.,

and at the expiration of that time examined both with the low and the medium power for blood shadows. Only such donors are accepted whose reciprocal specimens with the recipient's serum and corpuscles show no hemolysis. An occasional blood shadow may be disregarded, but the presence of a larger number, after as compared with before incubation, should cause the rejection of the donor in question.

Surgeons have largely been in the habit of accepting donors whose blood specimens showed agglutination when brought together with the recipient's blood. There can be no doubt, however, that this is irrational, and if at all possible only such donors should be accepted who show neither agglutination nor hemolysis. The occurrence of agglutination, to be sure, may not prove harmful to the patient, but the respiratory surface of the volume of blood affected is certainly smaller than in the absence of agglutination, and on this basis alone the custom of accepting "agglutinating donors" should be abandoned.

A hemoglobinemia, aside from the urinary examination, may be readily recognized by a spectroscopic examination of the serum, when the two bands of absorption of oxyhemoglobin will be observed.

A very simple method which may be employed for the same purpose is the following: One-half to 1 c.c. of blood is collected in a small glass tube, drawn out and sealed at one end. This amount can be readily obtained by puncturing the ear and milking out the blood. After the blood has clotted, the clot is separated from the walls by means of a wire or a glass rod and the corpuscles packed down by centrifugation. With normal serum the supernatant fluid presents a straw-yellow color, while in hemoglobinemia it is colored a more or less intense red. If the supernatant fluid is withdrawn, diluted with a little water, and heated to 70° to 80° C., the coagulum in the presence of hemoglobin will present a brownish color.

In some cases, and particularly in those following poisoning with chlorates, etc., the hemoglobinemia leads to a well-pronounced methemoglobinemia (see below).

Carbon Monoxide Hemoglobin.—In cases of coal-gas poisoning, the blood, both of arteries and veins, presents a bright cherry-red color, owing to the presence of carbon monoxide hemoglobin.

Such blood, when properly diluted, like oxyhemoglobin, shows two bands of absorption between *D* and *E* (Fig. 32), which are nearer the violet end of the spectrum, however, and may readily be distinguished from those referable to oxyhemoglobin by the addition of a reducing agent. This will not affect the spectrum of carbon monoxide hemoglobin, while that of oxyhemoglobin is transformed into the spectrum of reduced hemoglobin.

For medicolegal purposes a number of additional tests have been devised, among which that suggested by Hoppe-Seyler is one of the simplest and at the same time reliable. The blood is treated with double its volume of a solution of sodium hydrate (sp. gr. 1.3). Normal

blood is thus changed into a dirty-brownish mass, which exhibits a trace of green when spread upon a porcelain plate, while carbon monoxide blood yields a beautiful red under the same conditions.

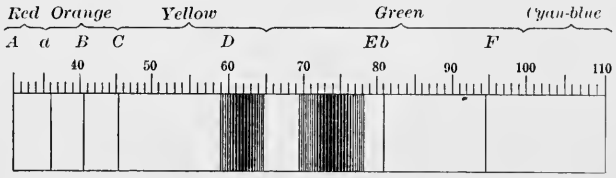


FIG. 32.—Spectrum of carbon monoxide hemoglobin. (v. Jaksch.)

Nitric Oxide Hemoglobin.—The blood in cases of poisoning with nitric oxide, owing to the presence of nitric oxide hemoglobin, yields a spectrum which is similar to that of carbon monoxide hemoglobin; the bands, however, are less sharply defined and paler than those of the latter, and, like these, do not disappear on the addition of a reducing substance.

Sulphohemoglobin (Methemoglobin Sulphide).—In cases of poisoning with hydrogen sulphide no definite changes can be discovered in the blood upon spectroscopic examination, although Hoppe-Seyler has shown that hemoglobin may enter into combination with this gas. It is stated, however, that in such cases the blood becomes dark and of a dull greenish tint, and that the distinction between arterial and venous blood is lost.

A remarkable instance of sulphohemoglobinemia has been described by v. d. Berg, in a case of autotoxic enterogenous cyanosis. In this case an organism producing hydrogen sulphide was isolated from the stools. When grown in a solution of normal oxyhemoglobin sulphohemoglobin resulted.

Carbon Dioxide Hemoglobin.—With carbon dioxide, as mentioned above, hemoglobin is also thought to enter into combination, the spectrum being similar to that of reduced hemoglobin. The latter in fact, is formed artificially when carbon dioxide is passed through a solution of oxyhemoglobin. If this process is carried further, the hemoglobin is decomposed and globin thrown down; an absorption band is then obtained which is similar to that resulting when hemoglobin is decomposed with acids (see below), and is no doubt referable to the presence of free hemochromogen.

Of the blood changes occurring in cases of poisoning with *hydrocyanic acid* and *acetylene* but little is known, and the reader is referred to works on toxicology for their consideration.

Hematin.—If oxyhemoglobin in aqueous solution is heated to a temperature of from 60° to 70° C., it is decomposed into globin and hematin. The same result is reached by treating the aqueous solution with acids, alkalies, or the salts of various heavy metals.

Hematin is an amorphous, blackish-brown, or bluish-black substance which is frequently encountered in old transudates, in the stools after hemorrhages, and after the ingestion of red meats in large amounts. It is said to occur in the urine in cases of poisoning with arsenic, and in the blood of animals poisoned with nitrobenzol its presence can likewise be demonstrated with the spectroscope.

In acid solution it shows a well-defined spectral band between *C* and *D*. Between *D* and *F* a second band is seen, which is much wider but less sharply defined than the first, and may be resolved into two bands by dilution, one between *b* and *F*, near *F*, and another between *D* and *E*, near *E;* a faint fourth band may also be seen between *D* and *E*, near *D*. As a rule, only the two bands between *D* and *F* are visible.

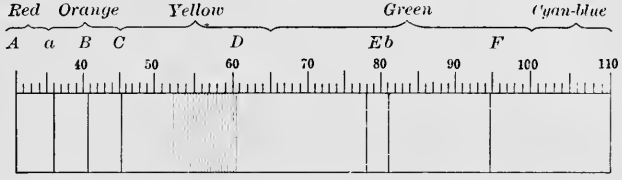


Fig. 33.—Spectrum of hematin in alkaline solution. (v. Jaksch.)

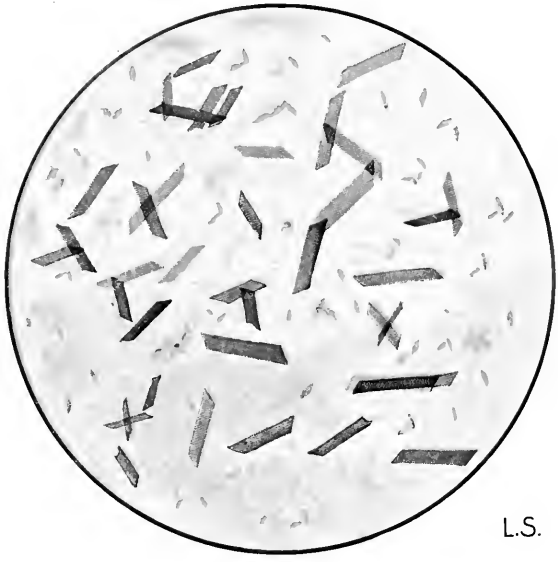
In alkaline solutions it shows but one broad band, the greater portion of which lies between *C* and *D*, extending slightly beyond *D* (Fig. 33).

If an alkaline solution of hematin is treated with a reducing substance, reduced hematin (hemochromogen) results, which gives rise to two absorption bands between *D* and *E* (Fig. 34).

Hemin.—Hematin readily combines with one molecule of hydrochloric acid to form hemin. This substance crystallizes in light brown or dark brown rhombic plates or columns, which are quite characteristic (Plate VII). They bear the name of their discoverer, Teichmann. The size of these crystals varies with the manner in which they are produced, the largest specimens being met with when the glacial acetic acid (see below) is allowed to evaporate as slowly as possible. Specimens measuring from 15μ to 18μ in length may then be seen. Smaller crystals will be present at the same time, occurring either singly or in the form of stars, rosettes, and crosses.

As these crystals may be obtained from mere traces of blood, their formation must be regarded as conclusive evidence in medicolegal examinations. Lewin and Rosenstein have pointed out, however, that under certain conditions a negative result may be reached, even if the coloring matter is derived from the blood. This is the case especially when the hemoglobin has been transformed into hemochromogen or hematoporphyrin, or when substances have been mixed with the blood which are either capable of altering its general com-

PLATE VII



Hemin Crystals.

position or which, through their mere presence, interfere with the reaction. Such substances are certain salts of iron (rust), lead, mercury, and silver; further, lime, animal charcoal, and sand, when intimately mixed with the blood. In medicolegal cases a spectroscopic examination should hence be made whenever the hemin reaction is not obtained.

METHOD.—A small drop of normal salt solution is slowly evaporated on a slide, when a few particles of the suspected material, powdered or teased as finely as possible, are placed on the delicate layer of crystallized salt. Glacial acetic acid is now added drop by drop and the specimen carefully heated (three quarters to one minute) until bubbles begin to form. While evaporation is being continued glacial acetic acid is further added until a light-brown tint appears. As soon as this point is reached, the last traces of the acid are allowed to evaporate, the specimen being held at a greater distance from the flame. A drop of glycerin is then added and the preparation covered with a cover-glass. The examination is made with a one-fifth or a one-sixth objective. Attention is especially directed to brownish streaks or specks, which, in the presence of blood, can usually be made out with the naked eye.

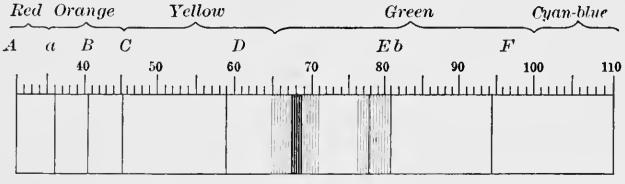


FIG. 34.—Spectrum of reduced hematin. (v. Jaksch.)

Methemoglobin.—Methemoglobin is a pigment closely related to oxyhemoglobin, and is frequently encountered in hemorrhagic transudates, cystic fluids, and in the urine in cases of hematuria and hemoglobinuria. In the circulating blood, methemoglobin is found after the ingestion of large quantities of potassium chlorate, notably in children, as also after the inhalation of nitrite of amyl, the use of kairin, thallin, hydroquinon, pyrocatechin, iodin, bromin, turpentine, ether, perosmic acid, permanganate of potassium, and antifebrin. (See Hemoglobinemia.) Most remarkable is the occurrence of methemoglobinemia in cases of so-called autotoxic enterogenous cyanosis, as reported by Stokvis and v. d. Berg. In one case the latter found sulphohemoglobin in the place of methemoglobin.

The spectrum of an aqueous or slightly acidified solution of methemoglobin (Fig. 35) closely resembles that of an acid solution of hematin, but differs from this in the ease with which it is transformed into that of hemoglobin when an alkali and a reducing substance are

added. The spectrum of hematin under the same conditions is transformed into that of an alkaline solution of hemochromogen. In alkaline solutions, on the other hand, two bands of absorption are observed, which are similar to those of oxyhemoglobin, but differ from these in the fact that the band nearer *E b* is more pronounced than the one at *D*, *a*. A third, but very faint, band may further be observed between *C* and *D*, near *D*.

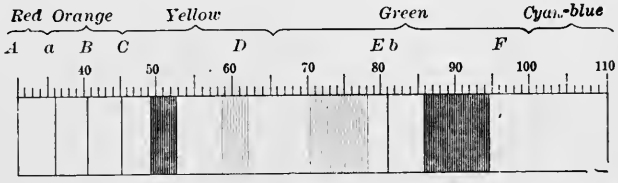


Fig. 35.—Spectrum of methemoglobin in acid and neutral solutions. (v. Jaksch.)

Hematoidin and Hemosiderin.—Small amorphous particles of an orange or ruby red color, or crystals belonging to the rhombic system, occurring either singly or in groups, are frequently met with in the sputum, the urine, and the feces, as well as in old extravasations of blood. They are in part iron containing—hemosiderin—or free from iron—hematoidin. Both are derivatives of hemoglobin, hematoidin being supposedly identical with bilirubin.

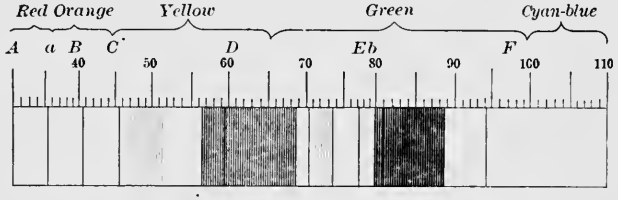


Fig. 36.—Spectrum of hematoporphyrin in alkaline solution.

Hematoporphyrin.—Hematoporphyrin is likewise a derivative of hematin, and, according to Nencki and Sieber, isomeric with bilirubin. In dilute solution with sodium carbonate it shows four bands of absorption, one between *C* and *D;* a second one, broader than the first, about *D*, especially marked between *D* and *E;* a third one, not so broad and less sharply defined, between *D* and *E*, and a fourth one, broad and dark, between *b* and *F* (Fig. 36).

The clinical significance of this body, which also appears in the urine, as well as the causes which give rise to its formation, are unknown (see Hematoporphyrinuria). It has been found post mortem in the blood, in a case of sulphonal poisoning, by Taylor and Sailer.

THE PROTEINS OF THE BLOOD

In considering the proteins of the blood from a clinical point of view, it is necessary to distinguish between an increase and a diminution in their amount, constituting the conditions of *hyperalbuminosis* and *hypalbuminosis*, respectively. As may be expected, the former is met with whenever water is more rapidly withdrawn from the system than it can be supplied, and is hence observed in cases of cholera, acute diarrhea, following the use of purgatives, etc. This increase in the amount of proteins is only a relative increase, however, and analogous to the corresponding polycythemia and hyperchromemia. The occurrence of an absolute increase has not been satisfactorily demonstrated. An absolute hypalbuminosis, on the other hand, is observed following a direct loss of proteins from the blood, as in hemorrhage, dysentery, albuminuria of high degree, the formation of large collections of pus, etc. This is generally associated with a relative increase in the amount of water—*i. e.*, a hydremia—which is particularly noticeable after hemorrhages, and referable to a diminished secretion and excretion of water, as well as to a direct absorption from the tissues. Hypalbuminosis has also been observed in pernicious anemia, and is dependent partly upon a diminution in the amount of the albumins of the serum and partly upon a decrease in the weight of the corpuscular solids. The amount of serum-albumin is about normal, while the globulins are much diminished. An increased content of globulins (hyperglobulinism) has been noted by several investigators in syphilis, and may be of diagnostic importance. Noguchi states that he has noted the increase in the globulin earlier than the presence of the syphilitic antibody, and that in cases of latent syphilis this may escape detection, whereas it is exceptional not to find the globulin increased. (See Part II, Syphilis.) He recommends the following method of testing for this increase:

Noguchi's Method.—One part of clear serum (0.5 c.c.), free from hemoglobin, is mixed with nine parts (4.5 c.c.) of a half saturated (neutral) solution of ammonium sulphate and centrifugated for thirty minutes in a machine which runs at a rate of 5000 revolutions a minute. The supernatant fluid is pipetted off. The deposit (which may be weighed) is dissolved in 10 parts (5 c.c.) of 0.9 per cent. salt solution. Of this solution, 1 part (0.5 c.c.) is mixed with an equal quantity of a 10 per cent. butyric acid solution. If the serum tested was of syphilitic origin a dense milky turbidity appears promptly, while the solution remains clear or shows only a slight opalescence without precipitation after several hours' standing, if it was derived from persons not suffering from syphilis. (See also the butyric acid test with cerebrospinal fluid.)

The term *hyperinosis* has been applied to a condition in which the amount of fibrin (normally 0.349 to 0.425 per cent.) is increased.

This is said to occur in various inflammatory diseases, such as pneumonia, pleurisy, scarlatina, acute articular rheumatism, and erysipelas, while a diminished amount of fibrin, *hypinosis*, or normal values are seen in malaria, nephritis, pyemia, pernicious anemia, typhoid fever, and leukemia (both lymphoid and myeloid).

In order to determine the amount of fibrin, 30 to 40 c.c. of blood, obtained by aspiration of a vein, are placed in a previously weighed beaker, fitted with an India-rubber cap, through the centre of which passes a piece of whalebone, firmly fixed. The blood is defibrinated by beating with the whalebone, when the beaker with its contents is weighed, the difference indicating the weight of the blood. The beaker is then filled with water and the mixture again beaten. The fibrin is allowed to settle and after being washed with normal salt solution collected on a filter of known weight. It is further washed with normal salt solution until free from coloring matter, then boiled in alcohol to dissolve out fat, cholesterin, and lecithin, dried at 110° to 120° C., and on cooling weighed over sulphuric acid.

Fairly satisfactory results may also be obtained by simply making wet mounts (which see), ringing with vaselin, and setting aside for several hours, when they are examined microscopically. In cases of pneumonia and acute articular rheumatism marked fibrin formation will be observed, starting from clumps of blood platelets.

The presence of *albumoses* and *peptone bodies* in the blood of leukemic (myeloid) patients has been repeatedly observed after the blood has stood for some time, or after the death of the patient (v. Jaksch, Matthes, Erben, Schumm). Their formation is due to the liberation of a proteolytic ferment, derived from the polynuclear neutrophiles. Schumm also found leucin and tyrosin. In normal human blood Schumm found no albumoses after death. In interstitial nephritis a fair amount could be demonstrated.

Albumoses have also been found in a case of abscess of the brain, associated with albumosuria. Freund claims that they are met with in sarcoma, while they are absent in carcinoma (not confirmed).

Following the injection of nuclein and spermin albumosemia appears to occur quite constantly, both during the stage of hypo- as well as hyperleukocytosis. After injections of pilocarpin, albumosuria is observed only in association with hyperleukocytosis.

In order to test for albumoses, the coagulable albumins should first be removed, when a positive biuret reaction in the filtrate will indicate their presence (see also Salkowski's test).

BLOOD SUGAR

Dextrose is a normal constituent of the blood, its quantity varying between 0.06 and 0.1 per cent. with 0.085 as an average.

A rise seems to occur physiologically just before or during the

menstrual period which is followed by a drop to normal or even subnormal values with the cessation of the bleeding; the variations between the highest and lowest readings, according to Kohler, amount to 0.03 per cent. in more than half the cases.

Temporary hyperglycemia may also be noted following the ingestion of large quantities of sugars and starches, in connection with emotional disturbances, and during pregnancy.

Under pathological conditions hyperglycemia has been observed in pancreatic and certain hepatic diseases, in connection with injury, tumor or hemorrhage affecting the brain, peripheral nerve lesions, but especially in maladies involving the endocrine glands and hence in diabetes when figures exceeding 0.4 per cent. are not uncommon. During coma the amount may indeed exceed 1 per cent.

Large quantities of a reducing substance, the greater portion of which consisted of sugar, have been met with by Trinkler in carcinoma; it was observed at the same time that carcinoma of internal organs was associated with far greater amounts of sugar than cancerous disease of the skin and the mucous membranes. It is also interesting to note in this connection that an increase in the degree of the cachexia was not accompanied by an increase in the percentage of sugar.

The results reached by Trinkler apparently also bear out the correctness of the conclusions formed by Freund, who claimed that a differential diagnosis between carcinoma and sarcoma, in which latter condition no increase in the amount of sugar was noted, can always be effected upon the basis of an examination of the blood in this direction. Further examinations on this point are lacking.

In addition to sugar, a non-fermentable reducing substance has been encountered in the blood, which, according to Mayer, appears to be a compound glucuronate. The presence of jecorin in the blood still remains to be proved.

The estimation of the sugar in the blood is best conducted according to Bang's method.

Bang's Method (modified by Thannhäuser and Pfitzer).—*Reagents Required.*—1. A copper solution prepared by dissolving 160 grams of potassium bicarbonate in from 600 to 700 c.c. of distilled water, adding 100 grams of potassium carbonate, 66 grams of potassium chloride and 100 c.c. of a 4.4 per cent. copper sulphate solution, and diluting to 1000 c.c. with water. The reagent is ready for use after standing for twenty-four hours.

2. A 20 per cent. solution of potassium chloride, which may be prepared by diluting 150 c.c. of a saturated solution with 70 c.c. of water.

3. A $\frac{N}{100}$ solution of iodin, which is prepared by adding 2 grams of potassium iodide and 1 c.c. of a 2 per cent. solution of potassium iodate to 5 c.c. of $\frac{N}{10}$ hydrochloric acid and diluting to 50 c.c. with water. As this solution is unstable it must be prepared anew every three or four days.

4. A 40 per cent. acetic acid solution.

5. A 1 or 2 per cent. solution of starch.

Apparatus Required.—1. Disks of blotting paper measuring about 10 by 20 mm.; the blotting paper is previously boiled in dilute acetic acid so as to remove traces of protein and sugar, and then dried.

2. A 50 c.c. Jena flask with a straight neck; flasks provided with side arms are especially convenient.

3. A carbon dioxide flask.

4. A 1 or 2 c.c. pipette reading in hundredths of a cubic centimeter.

Method.—Two or three drops of blood are received on a previously weighed blotting disk and the weight of the blood ascertained by a second weighing. To this end small weighing flasks will be found convenient.

7 c.c. of boiling hot potassium chloride solution are added as well as 2 or 3 drops of a 40 per cent. solution of acetic acid. The proteins are thus precipitated on the paper, while the sugar passes into solution. After a half-hour's cooling the solution is transferred to the Jena flask, using 4 c.c. of the potassium chloride solution for washing purposes. Three c.c. of the copper solution are now added when the mixture is boiled over a micro-burner for exactly two minutes, one minute and a quarter to a half being allowed to bring it to the boiling point. (This procedure should be practised first before proceeding to an actual test.)

After boiling a stream of carbon dioxide is allowed to enter the flask (not the liquid), to prevent reoxidation, and the latter immediately immersed in cold water (3 seconds). A few drops of the starch solution are then added as indicator when the amount of reduced copper is ascertained by titration with the iodin solution, the end-point being indicated as soon as the blue color of the copper solution has been replaced by the deep blue color of the starch and iodide.

The quantity of iodin used in the titration is divided by 2.2; the resulting figure, minus 0.01 indicates the number of milligrams of sugar present in the solution, from which the percentage, *i. e.*, the amount in 100 grams of blood is calculated.

The entire process occupies less than three-quarters of an hour.

With this method a large amount of work has already been done and it may not be out of place to reproduce some of the findings in table form (the data being taken from Hopkins).

Alimentary glycemia following the administration of 100 grams of glucose (1 hour later)	0.102 to 0.149
Same in diabetics (1 hour later)	0.261 to 0.402
Diabetes	0.152 to 0.306
Pneumonia	0.084 to 0.158
Pancreatic disease	0.081 to 0.119
Apoplexy	0.118 to 0.182
Carcinoma	0.074 to 0.181
Hyperthyroidism	0.067 to 0.140
Pituitary disease	0.078 to 0.117
Typhoid fever	0.103 to 0.147

Epstein's Method.—This method may also be employed and gives satisfactory results. 0.2 c.c. of blood is placed in a graduated test tube containing a drop of a 2 per cent. solution of sodium fluoride or sodium oxalate. The collecting pipette is rinsed two or three times with water, the rinsings added to the blood and water to the 1 c.c. mark. A saturated solution of picric acid is added to the 2.5 c.c. mark, when the mixture is well shaken and centrifugalized. One c.c. of the clear supernatant fluid is pipetted off, boiled down to two or three drops in a suitable test tube; 0.5 c.c. of a 10 per cent. solution of sodium carbonate is added and the mixture boiled to the point of crystallization. This takes place when the fluid has been concentrated to two or three drops and turns a brownish red. A few drops of water are added, the tube is warmed and the contents transferred to a calibrated tube accompanying the microcolorimeter that is used

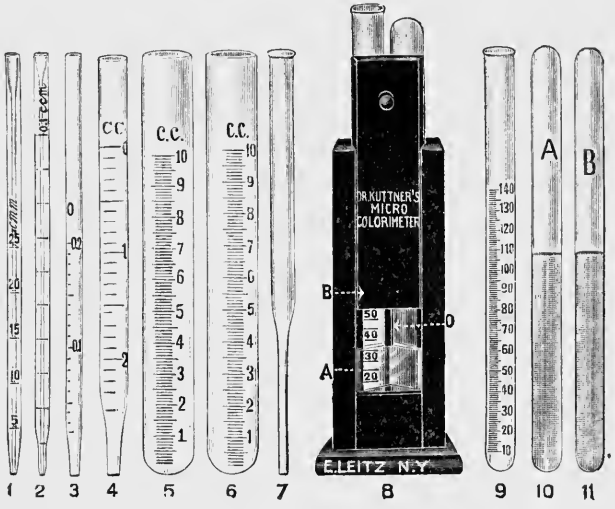


Fig. 37.—Kuttner's colorimeter and accompanying tubes and pipettes.

(Universal microcolorimeter of Kuttner-Leitz, Fig. 37).[1] The tube is rinsed with a few drops of water at a time and the total volume brought to the mark 50. The color is then compared with that of the two standard color tubes (A and B) which accompany the instrument. If it is darker than A, but lighter than B, the former is used as standard, water being added until the shades tally. The figure on the scale, divided by 1000, indicates the percentage amount of sugar in the blood. If the darker tube B is used for comparison, the figure reached must be multiplied by 2 and the result divided by 1000.

If the blood should contain 0.3 or more per cent. of sugar the process

[1] The same colorimeter can be used for hemoglobin estimations, as well as the permeation test and is marketed with the corresponding pipettes and standard color solution tubes.

should be repeated, using half the quantity of blood, viz. 0.1 c.c.; and it is accordingly best to charge two tubes at the start, the one with 0.2, the other with 0.1 c.c. of blood and the requisite amount of fluoride or oxalate, so that both quantities are available at the same time. The process is the same whether 0.1 or 0.2 c.c. be used, but in the former case it is necessary to multiply all obtained figures again by 2.

Example.—Tube B was used for comparison; the figure reached in the diluting tube was 75; 0.1 c.c. of blood was employed. Then $\frac{75 \times 2 \times 2}{1000} = 0.34$ per cent. would represent the amount of sugar present.

Glycogen.—There appears to be no doubt that glycogen normally occurs in the blood of various animals. Huppert succeeded in demonstrating its presence in all animals examined, the amount varying between 0.114 and 1.560 grams for 100 parts of blood (see Iodophilia).

Cellulose.—Cellulose has been found in the blood of tubercular patients.

UREA

The study of the urea content of the blood has attracted a good deal of attention of late and seems destined to occupy a permanent position in the laboratory diagnosis of renal insufficiency; this especially since simpler methods for its quantitative estimation have been devised, than were formerly available. The normal content of urea varies between 0.15 and 0.50 gram per liter of blood serum. Larger quantities are quite constantly found when the integrity of the renal epithelium is either temporarily or permanently damaged. Under such conditions the content may vary between 1 and 3 grams, the condition of the patient being the more precarious, the larger the quantity found, unless indeed the retention is due to a renal obstruction which is capable of removal. Widal states that patients whose blood contains 1 to 2 grams per liter rarely live more than a year, while in the presence of 2 to 3 grams death will probably occur within a few weeks or a few months, and with more than 3 grams even earlier. (See Nephritis.)

It is interesting to note that a smaller amount of urea is found in fatal cases of eclampsia than in those ending in recovery, which would indicate a deficiency in formation, as well as insufficient elimination.

Estimation of Urea.—Folin's Method.—Five c.c. of blood are obtained from one of the veins at the bend of the elbow by puncture with a hypodermic needle (1 mm. by 25 mm.) which has been connected with a 2 or a 5 c.c. pipette and this in turn with a rubber suction tube, carrying a clamp. Coagulation is prevented by putting a

small pinch of powdered potassium oxalate into the upper end of the pipette and allowing it to run into the tip of the needle. The blood is transferred to a 50 c.c. measuring flask half filled with pure methyl alcohol (free from acetone), which is then filled up to the mark. The mixture is shaken vigorously from time to time and after two hours or longer filtered through a dry filter. The filtrate is treated with two or three drops of a saturated alcoholic solution of zinc chloride and after a few minutes again filtered through a dry filter. 10 c.c. of the resultant filtrate correspond to 1.0 c.c. of blood. For a urea determination 10 c.c. of filtrate are used. This amount is placed in one of the large Jena test-tubes used in the total nitrogen estimation in urinary work (Folin's methods), treated with a drop of dilute acetic acid and two or three drops of kerosene. The tube is closed with a doubly perforated stopper. Through one hole passes a glass tube drawn out to a capillary several inches long, the capillary end reaching nearly to the bottom of the test-tube. Through the other hole passes a short bent glass tube which is connected with a good suction pump. The test-tube is placed in warm water and suction started to remove the alcohol. This is accomplished in from ten to thirty minutes. At the end of that time the capillary is broken off in the tube; 2 c.c. of 25 per cent. acetic acid are added, together with a temperature indicator (see index), a pebble and 7 grams of dry potassium acetate. The mixture is heated to a temperature of 153° to 158° C. at which the decomposition of the urea takes place in from eight to ten minutes, as described in the section on Folin's estimation of urea in the urine (which see). The ammonia is set free with an air current as usual, collected in a second large test-tube which has been previously charged with 1 c.c. of $\frac{n}{10}$ acid and 2 to 3 c.c. of water, Nesslerized (usually with only 3 c.c. of the diluted reagent, and diluted to 10 c.c., when the color comparison is made against a Nesslerized standard solution of ammonium sulphate (1 mg. of nitrogen) as described in the section on the urine. Since only 10 c.c. is available in the end, all of it must be poured into the Duboscq colorimeter cylinder for making the color comparison.

Marshall's Method.—This method is based upon the conversion of the urea of the blood into ammonium carbonate by means of the urease of the soy bean, and an estimation of the alkalinity before and after the conversion by means of standard acid and methyl orange. The method has the advantage that the proteins need not be removed.

Preparation of the Soy Bean Extract.—Soy beans are ground to a fine powder and stored in a well-stoppered dry bottle, when they will keep for months without appreciable loss of activity. From this stock product an extract is prepared anew every five or six days, by treating 10 grams with 100 c.c. of water and allowing this to stand with occasional agitation for one hour, when 10 c.c. of $\frac{n}{10}$

hydrochloric acid are added and the mixture is allowed to stand for about fifteen minutes longer. It is then filtered, covered, and shaken with toluene, and thus keeps its activity for the period of time designated.

The *blood* may be procured a day or two before the test is made, but should be kept on ice until the serum is separated by centrifugation.

Method.—Two equal portions of the serum (from 3 to 10 c.c. each) are placed in ordinary test-tubes. One tube receives 1 c.c. of the soy bean extract, and both are covered with a little toluene (0.5 to 1.0 c.c.). The tubes are tightly stoppered and allowed to remain at room temperature, until the conversion of the urea is complete. This usually takes about four or five hours; but it is convenient to let the tubes stand over-night. The contents of the tube containing

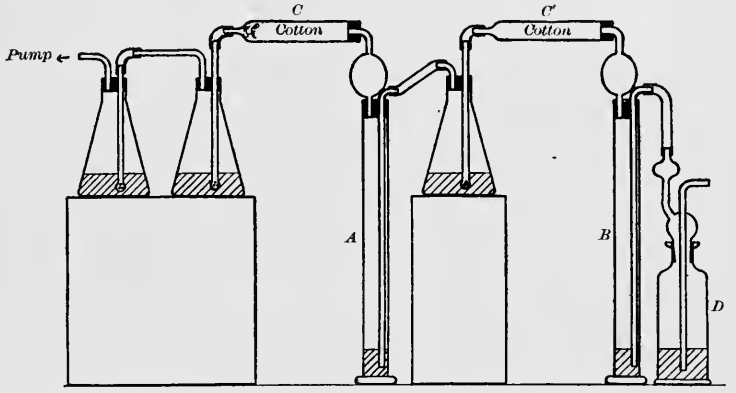


Fig. 38.—Marshall's method of estimating urea in the blood.

the serum and extract are transferred to cylinder *A* (Fig. 38) by the aid of a little water (not more than 5 c.c.). An equal volume of alcohol, two grams of sodium chloride and a layer of kerosene oil are added. Cylinder *B* is charged with the contents of the other tube and treated like *A;* 25 c.c. of $\frac{n}{50}$ hydrochloric acid and about 25 c.c. of water are placed in each of the 200 c.c. Erlenmeyer flasks used for the absorption of the ammonia; 0.5 gram of sodium carbonate is finally added to each cylinder when an air current is passed through the apparatus for one hour as with Folin's technique. The excess of the acid in the absorption flask is finally titrated with $\frac{n}{50}$ sodium hydrate using alizarin sodium sulphonate as indicator, when the corresponding amount of ammonia is estimated in the usual manner. The quantity of ammonia obtained from *B* is deducted from *A*, the difference being referable to the ammonia derived from the urea (1 c.c. $\frac{n}{50}$ HCl = 0.0006 gram of urea).

Precautions.—The absorption tubes dipping into the Erlenmeyer flasks are closed at one end and pierced with six or seven small holes as suggested by Folin. Two absorption flasks are used in connection with cylinder *A*, and one with *B*.

A layer of toluene is placed in the absorption flasks to prevent foaming. The bottle *D* contains dilute sulphuric acid, to free the air current from any ammonia that may be present.

Marshall's method as just outlined recommends itself especially owing to its simplicity, and seems to compare favorably with that of Folin.

THE TOTAL NON-PROTEIN NITROGEN OF THE BLOOD

Farr and Austin regard Folin's urea method (described above) as less reliable in studying increased nitrogenous retention than his method for the estimation of the total non-protein nitrogen. Working with this latter (see below) they found from 16 to 43 mgs. per 100 c.c. in a group of cases suffering from a variety of acute and chronic diseases, but without evidence of disturbance of renal function. In these the ammonia urea fraction was from 50 to 60 per cent. of the total. In patients with cardiovascular disease with renal congestion, but without evidence of other renal lesion, they obtained no increase of the non-protein nitrogen of the blood, nor alteration of the ammonia-urea percentage, although albuminuria, casts, and some impairment of the phenolsulphonephthalein (see permeation test) elimination were usually present. In that type of chronic nephritis characterized by marked albuminuria, cylindruria and edema there were similar findings. In chronic nephritis associated with hypertension, on the other hand, the non-protein nitrogen was markedly increased (40 to 181 mgs. per 100 c.c.), and the percentage of the ammonia-urea was usually higher than in the non-nephritic cases. Farr and Austin found that the nitrogen values in these cases were subject to rapid fluctuation in the course of a few days and that clinical improvement was associated with a fall in the non-protein nitrogen content. Uremia was almost always accompanied with some increase of the non-protein nitrogen, but no constant relation could be established between the degree of increase and the tendency to uremia.

Method of Folin.—The blood is secured and precipitated with alcohol as described sub "Urea in the blood" (see above), when 10 c.c. of the filtrate are transferred to a large Jena test tube as in the estimation of the total nitrogen of the urine (which see). One drop of sulphuric acid, one of kerosene, and a pebble are added and the methyl alcohol driven off by placing the tube in a beaker of boiling water for ten minutes. After removal of the alcohol 1 c.c. of

concentrated sulphuric acid, 1 gram of potassium sulphate and a drop of a 5 per cent. copper sulphate solution are added and the mixture boiled, cooled, and diluted as in the analysis of the urine. From this mixture the ammonia is removed as there described, but collected in a second large test-tube previously charged with 1 c.c. of $\frac{n}{10}$ acid and 2 to 3 c.c. of water, The resultant product is then Nesslerized by the addition of 7 to 8 c.c. (or more, if much ammonia is present) of diluted Nessler's reagent (dilution 1:15), and the mixture transferred to a measuring flask and diluted to 25, 50 or 100 c.c. with distilled water, the standard of comparison being as in the case of the urine, a Nesslerized solution of ammonium sulphate containing 1 mg. of nitrogen in 100 c.c. Here as there the colorimeter prism of the standard is set at 20 mm. (Duboscq).

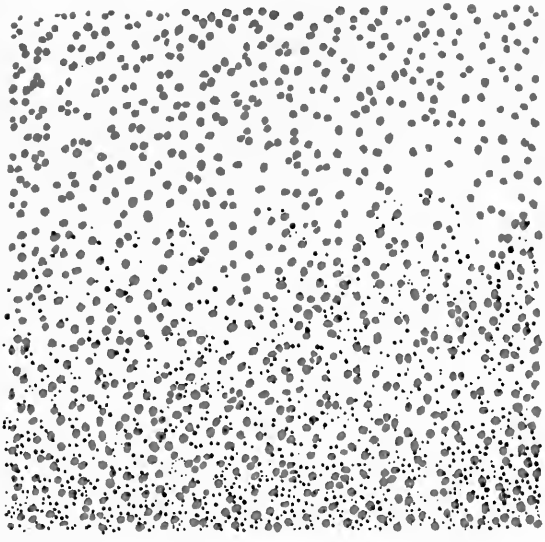
If 5 c.c. of blood has been used and 10 c.c. of the filtrate were diluted to 50 c.c. the calculation is made according to the formula $\frac{20 \times D}{R}$ in which R stands for the reading of the unknown, and D represents the volume to which the ammonia has been diluted.

AMMONIA, URIC ACID, AND XANTHIN BASES

Ammonia.—Normal venous blood, according to the researches of Winterberg, contains about 1 mg. of ammonia for every 100 c.c. In febrile conditions variable results are obtained, but it appears certain that a definite relation does not exist between the height of the fever and the amount of ammonia. In chronic hepatic diseases, and notably in cirrhosis, it is not increased. Acute yellow atrophy also is not necessarily associated with an increase. Very significant is the observation that in uremia following extirpation of the kidneys no increase is observed. Large amounts of ammonia are met with in diabetes and other conditions associated with acidosis. (For the estimation of the free ammonia see Determination of Urea.)

Uric Acid.—The uric acid content of normal blood is much greater than was formerly supposed, viz., 1 to 2.5 mgrm. per 100 c.c. The difference between the normal and gouty blood, as Folin has pointed out, is materially smaller than earlier investigations tended to show. In a study of twelve cases of gout he obtained values varying from 3.1 to 5.4 mgrm. per 100 c.c. with a purin-free diet for at least two days before the blood was taken. Since equally large amounts may be found in non-gouty conditions, such as arthritis deformans, gonorrheal arthritis, chronic nephritis, uremia, pneumonia, acute and chronic nephritis, leukemia, conditions associated with an insufficient aëration of the blood, as in the various diseases of the heart, in pleurisy with effusion, emphysema when accompanied by cyanosis, the severer forms of anemia, etc., the diagnosis of gout can evidently not be made upon the basis of individual blood examinations. Folin has pointed

PLATE VIII



Pronounced Lipemia. (Gumprecht.)

Specimen treated with osmic acid. Lower half shows extracellular fat globules, upper half having been cleared by oil of turpentine.

out, however, that whereas in these non-gouty conditions the non-protein-nitrogen is simultaneously increased (59 to 288 mgrm.), normal values are the rule in the gouty cases (28 to 42 mgrm.). Both estimations must, however, be made and it is indispensable, moreover, that the patient should have been on a purin-free diet for at least two days.

Xanthin Bases.—Xanthin bases do not occur in normal blood or are present only in exceedingly small amounts. Under pathological conditions they may be encountered in recognizable quantities, so in leukemia, typhoid fever, lymphatic tuberculosis, emphysema, phthisis pulmonalis, pleurisy, and chronic nephritis.

For a consideration of the methods used in estimating the amount of uric acid and xanthin bases, the reader is referred to works on Physiological Chemistry.

FATS, FATTY ACIDS, AND CHOLESTERIN

Fats and Fatty Acids.—Engelhardt has pointed out that the amount of *fat* which is contained in normal human blood may be subject to considerable variations, and gives 0.194 per cent. as the average. The lowest figure which he obtained was 0.101 and the highest 0.273 per cent. These figures differ very materially from those of older observers, who have found from 0.73 to 1.4 per cent., but it is quite likely that Engelhardt's method is responsible for these differences, and is probably more reliable (see below). Unfortunately only a few analyses of pathological material have been made with this method, and these have reference only to the blood of cachectic individuals. An increase in the amount of fat has here not been demonstrated, the results varying between 0.112 and 0.284 per cent., with 0.174 as an average. The cachexias in question were of tubercular and carcinomatous origin. With the older methods an increase in the amount of fat, aside from that observed after the ingestion of large amounts of fatty food, has been met with in cases of obesity, chronic alcoholism, in phosphorus poisoning, in injuries affecting the long bones and the spinal cord, in various hepatic diseases, chronic nephritis, tuberculosis, malaria, cholera, during starvation, pregnancy, in nursing infants, etc. The greatest increase, however, is observed in certain cases of severe diabetes, in which amounts varying between 1.276 and 18.12 per cent. have been encountered, and in which the fat may be visible with the naked eye (see below). This increase in the amount of fat constitutes the condition spoken of as *lipemia* (Plate VIII).

The term *lipacidemia* has been applied to the occurrence of fatty acids in the blood. This has been noted in various febrile diseases, leukemia, and especially in grave cases of diabetes, where beta-oxybutyric acid may be found in large amounts, and is no doubt directly concerned in the production of coma.

To demonstrate the presence of fat in the blood, it is best to prepare cover-glass specimens, and to mount these in a drop of a 5 per cent. solution of osmic acid. The fat droplets are thus colored black, and appear about as large as the finest fat granules which are found in milk or butter. They may also be stained with Sudan III, or Biebrich scarlet, and are thus colored red. In every case the necessary instruments and glasses should be carefully cleansed with ether, so as to avoid the accidental introduction of fat.

As a quantitative estimation of the fat is not always possible, Landy recommends the following simple procedure to demonstrate the presence of an excess of fat: A small drop of blood is received upon a cover-glass, which is then adjusted over the depression of a cupped slide and ringed with vaselin. On standing, the serum separates out concentrically or excentrically from the small blood clot, and normally or in the presence of no excess of fat appears perfectly clear. If, however, much fat is present, it becomes cloudy after several minutes or hours, and then appears bluish white, grayish white, or even milky white. To ascertain positively that the turbidity is due to fat, a microscopic examination of the hanging drop is made within a few hours following the preparation of the specimen, so as to exclude fibrin as the possible cause of such turbidity. (For a consideration of the quantitative method for the determination of fat in the blood, see special works on Physiological Chemistry.)

The fatty acids may be estimated along the same lines as described in the Urine (Lipaciduria), after removal of the coagulable albumins. At least 20 to 30 c.c. should be available.

Cholesterin.—Traces of cholesterin are normally met with in the blood. Larger amounts have been observed in diabetes (0.478 per cent.) in association with marked lipemia. Using a special (biological) method, I have demonstrated that the cholesterin content of the blood is diminished in many cases of tuberculosis and of syphilis.

Hale White reports a case in which microscopic examination showed a granular precipitate, which did not stain with osmic acid. Chemical examination led to the conclusion that the substance was an ester of cholesterin with one or more of the higher fatty acids.

LACTIC ACID

There appears to be some doubt whether or not lactic acid normally occurs in the blood of man during life. In the blood of dogs Gaglio could always demonstrate the presence of the acid during the process of digestion, after feeding with meat. The amount varied between 0.3 and 0.5 pro mille. During starvation smaller amounts were found, but it never disappeared altogether. In one instance Gaglio obtained 0.17 pro mille after fasting for forty-eight hours.

Similar results were obtained by Irisawa, who noted that the amount of lactic acid in the blood stood in direct relation to the degree of anemia which was produced.

In the human being Irisawa found lactic acid fairly constantly after death, the amount, determined as zinc lactate, varying between 0.233 and 6.575 pro mille. These extensive variations he was unable to explain by the character of the disease causing the fatal termination, and it is possible that the cause lies in the fact that in some cases the blood was obtained shortly after death, while in others many hours had elapsed, as Irisawa himself suggests. (For a consideration of the methods employed, see special works on Physiological Chemistry.)

HOMOGENTISINIC ACID

Homogentisinic acid has been demonstrated in the blood serum of an alkaptonuric, by Abderhalden and Falta.

BILIARY CONSTITUENTS AND UROBILIN

Bile pigment does not occur in the blood under normal conditions, but may be demonstrated whenever it is present in the urine (obstructive jaundice, hepatic cirrhosis, acute yellow atrophy, phosphorus poisoning, etc.). It appears, moreover, that bilirubin is present in the blood in nearly every case where urobilin is found in the urine. In pernicious anemia bilirubinemia is thus quite constantly associated with urobilinuria. At the same time urobilin can usually be demonstrated in the blood. In chlorosis bile pigment does not occur in the blood.

The demonstration of bilirubinemia constitutes the most delicate test for the entrance of bile into the blood and lymph; it is a much more delicate indicator than the occurrence of bilirubinuria.

Bilirubin can be demonstrated in the blood most readily in the following manner: 0.5 c.c. of blood, obtained from the finger or the ear, is collected in a small glass tube, and the serum separated from the corpuscles by centrifugation. The supernatant fluid is normally clear or but faintly turbid, and of a straw color; in the presence of bilirubin it is colored a bright yellow, and on exposure to the air this gradually turns to a greenish tint.

For more exact information the method of Syllaba may be used: 10 to 15 c.c. of blood are placed in a cool place for sedimentation. The serum which separates out is removed with a pipette and 5 c.c. diluted with double the amount of water and coagulated by boiling after the addition of a pinch of sodium sulphate and acidifying with dilute acetic acid. Any bilirubin that may be present is carried down in the coagulated albumin while urobilin remains in solution. The fluid is then filtered and the filtrate tested by boiling to make sure that the coagulation is complete.

If no urobilin is present the filtrate is clear, colorless, and spectroscopically free from absorption; if, however, urobilin is present in the serum, as is usually the case in pernicious anemia, then the filtrate presents a reddish color and shows a narrow band of absorption between *b* and *F*. The collected precipitate in the absence of bilirubin (in normal serum and the serum of chlorosis) is white, but in the presence of bilirubin (in the serum of pernicious anemia) of a slight yellowish color. The precipitate is washed with hot water, boiled with acidulated alcohol (sulphuric acid) and the mixture filtered. In the presence of bilirubin the alcohol is colored a fine green and the coagulum presents the same color; in the absence of bilirubin the alcohol remains colorless. (For a consideration of the demonstration of bile acids in the blood, the reader is referred to works on Physiological Chemistry.)

ACETONE

Acetone has been found in the blood in considerable amounts under various pathological conditions, and especially in diabetes and fevers.

In order to demonstrate its presence, *Denigè's test* may be employed: 3 c.c. of blood are treated with about 30 c.c. of Denigè's reagent (see urine) and allowed to stand until the dark brown precipitate has settled to the bottom. The supernatant fluid is filtered off and treated with a little more of the reagent, so as to insure *complete* precipitation. It is then acidified with sulphuric acid and heated as described in the section on the Urine. The formation of a white precipitate, which is soluble in an excess of hydrochloric acid, is referable to acetone or diacetic acid.

CHOLIN

Cholin has been demonstrated by Moth and Halliburton in the blood in diseases of the nervous system which are associated with a destruction of nerve tissue; notably in *general paresis*, *tabes*, combined sclerosis, disseminated sclerosis, alcoholic polyneuritis, beriberi, and following the division of both sciatic nerves in cats.

Method.—Five c.c. of blood are treated with from six to eight times that amount of absolute alcohol and filtered. The filtrate is dried at 40° C., and the *dry* residue extracted three times with *absolute* alcohol, filtered, and the solution evaporated. The alcoholic solution of the residue is precipitated with a 10 per cent. alcoholic solution of platinum chloride and the precipitate decanted from the absolute alcohol. The precipitate is finally dissolved in 15 per cent. alcohol, the solution filtered and evaporated in a watch crystal at 40° C. With a low power the octahedral crystals of cholin-platinochloride can then be seen.

Normal human blood (in the amount mentioned) rarely gives rise to such crystals, so that the result is practically negative. *Sine qua non* for the success of the method is that the alcohol is absolute; 99 per cent. will not suffice. (See also Donath's method, Sub-cerebrospinal Fluid.)

INDICAN

According to Obermeyer and Pepper a marked increase in the amount of indican in the blood is practically diagnostic of uremia. This view is confirmed by the findings of Dorner.

Method.—10 to 20 c.c. of blood are precipitated with five times its volume of 95 per cent. alcohol and filtered. The alcoholic filtrate is evaporated, the residue taken up in water, two or three drops of a strong lead acetate solution added and the filtrate made up to 10 c.c. by the addition of water. This is treated with an equal volume of Obermeyer's reagent (see Urine) and the mixture shaken in a water-bath with 1 c.c. of chloroform. A more or less intense blue in the chloroform indicates the presence of an abnormally large quantity of indican.

It is noteworthy that marked indicanuria, unaccompanied by renal disease, is not associated with the presence of an increased amount in the blood.

KRYOSCOPIC EXAMINATION OF THE BLOOD

The kryoscopic examination of the blood has for its object the determination of the molecular concentration, and hence of the osmotic pressure of the blood. The method is essentially based upon the observation of Raoult: (*a*) That all solid, liquid, or gaseous substances when dissolved in a liquid will lower the freezing point of that liquid; (*b*) that the degree to which the freezing point is lowered is dependent upon the amount of substance which is present in solution; and (*c*) that equimolecular solutions have like freezing points. It follows that the freezing point of a solution furnishes an index of its molecular concentration, and hence also of its osmotic pressure, as this has been shown by van't Hoff to be proportionate to the number of molecules present.

The degree to which the freezing point is lowered is designated by the letter *J*. In the case of normal blood this varies between —0.56 and —0.58° C., as compared with distilled water. A further depression is probably always indicative of renal insufficiency. A study of this symptom is of special value in the domain of renal surgery. As the result of 265 freezing-point determinations of the blood, in 170 cases in which various operations were performed upon the kidney and in which a direct examination of the organ was possible, Kümmel concludes that kryoscopy furnishes a more reliable index of

renal insufficiency than any other method. Other observers, such as Casper and Richter, Tinker, and others, have arrived at similar conclusions. To Koranyi, however, belongs the credit for the introduction of kryoscopy into the clinical laboratory and its application to the study of renal diseases. Senator, Claude, and Balthazar, Albarran, Kövesi, Lindemann, Waldvogel, and others have materially contributed to establish its value as a clinical method.

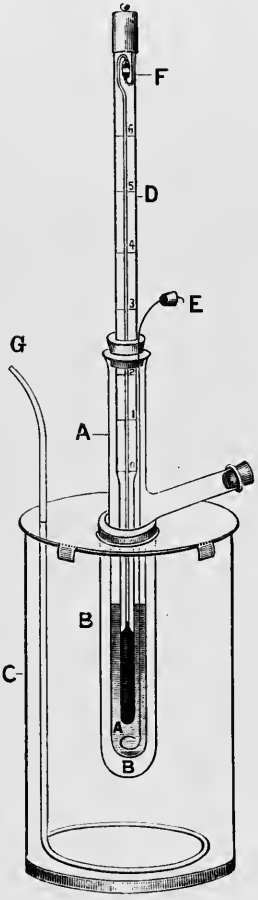


Fig. 39.—Beckmann's apparatus.

Zangemeister, who has carefully studied the molecular concentration of the blood during pregnancy, the puerperal period, and in eclampsia, found a lessened concentration in the first instance, and values in the second which were still below the normal average and yet slightly higher than in pregnancy. In eclampsia the average concentration was normal. Similar results have been obtained by others, such as Füth and Krönig, Szili, and Lobenstine. During pregnancy (ninth month) the latter found the average Δ in 12 cases to be —0.51° (variations from 0.45° to 0.57°); the average value in 12 puerperal women was —0.53° (variations: —0.49° to —0.58° C.). He accordingly concludes that if there is retention in eclampsia it must be of either colloidal substances or of crystalline substances, too small in amount to affect the concentration of the blood.

Schmidt has recently studied the kryoscopic behavior of the blood in pneumonia, and as the result of an analysis of 24 cases he concludes as follows:

There is an absolute lowering of the freezing point in pneumonia, which seems to depend either on the extent of the consolidation or on the height of the temperature or on both. The concentration of the blood increases, as shown by the lowered freezing point, as the disease progresses up to the time of the crisis. In those cases where the heart weakens perceptibly the freezing point of the blood becomes lower and in the fatal cases in which the heart gives out the freezing point is very low.

Method.—In the clinical laboratory a modification of Beckmann's apparatus is most conveniently employed (Fig. 39). Its essential parts are: a Heidenhain thermometer (*D*) graduated in hundredths

and reading from —1° to —5° C.; a platinum wire loop for stirring (*E*); a test-tube (*A*) which is closed by a stopper through which the thermometer and stirring wire pass, and which in turn is placed in a second larger tube (*B*) so as to be surrounded by an air space. The jar (*C*) is filled with a freezing mixture of salt and ice, the temperature of which should lie between —2° and —5° C. Into this is placed the second tube *B*. The test-tube *A* is charged with 20 c.c. of blood (if only 10 c.c. are available, this amount may suffice), obtained by means of a large aspirating syringe from one of the veins near the bend of the elbow; the thermometer is introduced and the stirring wire adjusted. The tube is placed directly in the freezing mixture until the mercury leaves the reservoir bulb (*F*); this is done to save time. It is then adjusted in the second tube, as shown in the illustration, and the blood constantly stirred with the platinum wire. The temperature falls more or less rapidly below the freezing point before actual freezing takes place; as this occurs it suddenly rises again owing to liberation of heat, and then remains constant for some time. This point represents the true freezing point. Later, if the tube is allowed to remain in the freezing mixture, the temperature may fall to that of the latter. The difference between the freezing point of distilled water and that of the blood is *Δ*.

In every case it is necessary to determine the true zero for each instrument separately, as this often varies somewhat owing to unavoidable errors incident to its construction. To this end the tube *A* is charged with three to four times the amount of distilled water which is necessary for one examination The greater portion of this is frozen; the liquid portion is thrown away; the frozen water is allowed to thaw and is again frozen in part, a portion being again thrown away; the remainder is sufficiently pure for the examination.

The freezing mixture is prepared by packing alternate layers of ice and salt into the jar around the tube *B*, which is held in position while the ice is packed. Ice and salt are finally thoroughly mixed by stirring with a heavy wire ring and rod (*G*). If several examinations are to be made, the water which separates out is poured off and replaced by an additional amount of salt and ice.

The method is quite expeditious, and if everything is previously prepared the examination does not occupy more than ten or fifteen minutes.

STUDY OF THE OSMOTIC RESISTANCE OF THE RED CELLS

Janowsky's Method.—The red cells are first counted as usual, using a 3 per cent. solution of sodium chloride as diluent. Then a second count is made, this time with a hypotonic (0.4 per cent.) salt solution and a dilution of 1 to 200. Ten minutes should be allowed to elapse before mounting the drop. At the end of this time even

normally a certain number of red cells lose their hemoglobin. This number is expressed in percentage terms, pro 1 c.mm. of blood. The examination should always be made upon an empty stomach, and in accurate work the barometric pressure and in cases of heart disease the height of the blood pressure should also be taken into consideration.

Under normal conditions the corpuscular stability is subject to definite individual variations, which lie within very narrow limits. It is increased by physical and mental labor, diminished by baths and diet free from meats.

Jakuschewsky found normal values in diabetes (excepting in coma), pseudoleukemia, the primary stages of syphilis, chronic gastritis, atrophic hepatic cirrhosis, subacute parenchymatous nephritis, pyelonephritis, hysteria, and minor chorea. In aortic aneurysm the stability is high, but quite analogous to what is found in normal old people with physiological sclerosis.

Increased stability associated with an increase in the severity of the clinical symptoms and *vice versa* was noted in the following conditions: Typhoid and typhus fever, recurrens, croupous pneumonia, acute and chronic malaria, influenza, acute rheumatism, advanced pulmonary tuberculosis, intestinal tuberculosis; chronic parenchymatous and interstitial nephritis (in association with uremic symptoms); anemia, chlorosis, leukemia, catarrhal jaundice; Charcot-Hanot's (biliary) cirrhosis; attacks of cholelithiasis with bile retention; acute gout; myocarditis (with beginning insufficiency); organic heart disease during lack of compensation; the final stage of carcinoma of the stomach.

Jakuschewsky thinks that the determination of the corpuscular stability may be of prognostic significance—an increase or retarded diminution *cæteris paribus* indicating an aggravation of the condition—and at times also of diagnostic value (carcinoma of the stomach).

THE BACTERIOLOGICAL EXAMINATION OF THE BLOOD

The blood is most conveniently collected in large vacuum tubes (Plate IX) resembling the common Keidel tube, and containing glucose bouillon with 1.5 per cent. sodium citrate, so as to prevent coagulation. The technique is very simple. The skin at the bend of the elbow is cleansed with alcohol. A tourniquet or similar contrivance is placed about the upper arm, and the needle of the vacuum tube thrust into any one of the large veins which present, care being taken to enter the vein parallel to its longitudinal course. The capillary end of the tube within its rubber cover is knicked off, when the blood will be seen to enter the bulb. From 3 to 5 c.c. for from 20 to 25 c.c. of the culture medium is sufficient. The tourniquet is removed, the needle withdrawn, and the outer guard tube immedi-

PLATE IX

Blood Culture Tube.

ately replaced, when the entire outfit is placed in the incubator and observed for a number of days. The corpuscles rapidly settle, and the supernatant becomes clear, if no growth develops, while a turbidity results sooner or later, if living organisms are present. In the case of the typhoid bacillus, growth usually occurs within 24 to 36 hours, while, with certain streptococci, it is necessary to incubate for a week before any definite statement can be made. After growth has taken place transfers may be made for purposes of more detailed study, but as a rule morphological examination of a drop of the turbid culture medium suffices for general identification.

If anaërobic conditions are desired tubes are employed which have been covered with liquid paraffin and which may subsequently be exhausted again and then sealed.

If the specimens are to be sent through the mail they are sealed, while ordinarily this is not necessary.

Using this method in my laboratory during the past few years we have obtained most satisfactory results.

If the number of organisms per c.c. of blood is to be ascertained plating is of course necessary, in which case the older methods must be employed, viz., withdrawal of blood with a syringe and inoculation of agar tubes with a measured quantity (one part of blood to about five of agar) followed by plating.

A study of the bacterial findings in the different infectious diseases shows that the corresponding bacteria may be found in the blood in practically all. There is a considerable difference in the frequency, however, with which organisms appear in the individual cases. These points will be considered in some detail in the second part of the book, under the headings of the special diseases. At this place the bacteria in question are merely enumerated, precedence being given to those which are most frequently encountered in the corresponding infections: The typhoid and paratyphoid (paracolon) bacillus, the plague bacillus, the Micrococcus melitensis, the pneumococcus, Streptococcus pyogenes, Staphylococcus aureus, the gonococcus, meningococcus, anthrax bacillus, colon bacillus, proteus, Bacillus pyocyaneus, Micrococcus zymogenes, the tubercle bacillus, the bacillus of leprosy, the influenza bacillus, Friedländer's bacillus, Micrococcus tetragenus, the gas bacillus, the diphtheria bacillus. In addition to these organisms Oidium albicans, pathogenic blastomycetes and streptothrix have been encountered in isolated cases. The spirillum of relapsing fever, which is so uniformly found in the disease in question, is now classed among the animal parasites. A description of the different organisms will be found in the bacteriological section of the book.

On rare occasions bacteria have been found in the blood of patients directly upon microscopic examination. In meningococcus infections the corresponding organism has thus been repeatedly encoun-

tered. I have found it twice in a series of about a dozen cases; in one of these I estimated the number at 7,380,000 per c.c., almost all of which were enclosed in polynuclear neutrophiles and in large mononuclear elements which I looked upon as endothelial cells.

The anthrax bacillus also has been met with in direct microscopic examination. As a rule, the number is small.

Rosenberger a short time ago announced that the tubercle bacillus could frequently be demonstrated directly in the blood on staining the residual material after centrifugalizing a large amount of blood and destroying the red corpuscles. The study of others suggests, however, that the findings were erroneous and owing to the presence of acid-fast bacilli in the reagents employed.

THE PARASITOLOGY OF THE BLOOD

MALARIA

Malarial fever is referable to infection with a protozoan parasite belonging to the class of hematozoa, representatives of which are found in the blood of various animals, such as the rat, frog, turtle, carp, various birds, etc. Three varieties are known to occur in the blood of man, viz., the parasite of tertian fever—*Plasmodium vivax;* the parasite of quartan fever—*Plasmodium malariæ*, and the parasite of estivo-autumnal fever—*Plasmodium immaculatum*, vel *Laverania malariæ*. The life history of these organisms is now well understood, and it is known that in addition to the intracorporeal cycle of development which takes place in the human body there is yet another, an extracorporeal cycle, which occurs in mosquitoes of the genus Anopheles (Fig. 38). Infection occurs through the bites of such mosquitoes, which themselves have been infected by sucking the blood of malarial patients. This has been abundantly demonstrated by Ross, Manson, Grassi and others, and may be regarded as an established fact.

Method of Examination.—When the patient is directly available at the laboratory, or if a few hours only need elapse before the examination is made, wet mounts may be used, which are best ringed with a little vaselin or paraffin, if they cannot be examined at once. Otherwise, dry mounts are prepared and stained with the eosinate of methylene blue, or one of the Romanowsky dyes, such as Hastings', Wilson's, Wright's, Giemsa's, etc. (Plate XII.) With Romanowsky mixtures, which all contain methylene azure, the chromatin (nuclear) granules are shown.

It is best to procure specimens shortly before an attack, as adult forms are then obtained; immediately after an attack is not the proper time to hunt for parasites.

In cases in which but few organisms are expected Ross has suggested the advisability of spreading thick blood specimens and extracting

the hemoglobin before staining. The search for the youngest forms of the estivo-autumnal parasite especially is much facilitated in this manner. Ruge indorses this method in the following modification. A large drop of blood (about 20 c.mm.) is spread over a surface measuring about 18 square millimeters. The air-dried preparation is then placed for a few minutes in a 5 per cent. solution of formalin, to which 0.5 to 1 per cent. of acetic acid has been added. In this manner the hemoglobin is all extracted, while at the same time the blood film is fixed, so that it can now be washed without fear of ruining the preparation. It is then stained either according to one of the modifications of the Romanowsky method or with the eosinate of methylene blue. Ruge further advises that specimens stained according to the Romanowsky method be subsequently stained with Manson's solution,[1] in order to render the smallest and medium-sized ring forms more readily visible, as their affinity for the dye is somewhat impaired by the fixation in formalin. My own experience with this method has been very satisfactory.

Plasencia suggests the following method: Fixation in 0.5 per cent. formalin and absolute alcohol (equal parts); *rapid* drying in the air and washing in distilled water. The specimens are then stained with a mixture composed of 80 c.c. of a saturated aqueous solution of toluidin blue and 60 c.c. of 1 per cent. aqueous solution of eosin. After washing in water they are dried and examined as usual. Plasencia regards this stain as better than Manson's.

The Parasite.—The following forms of the parasite may be found in the blood:

1. **Hyaline Non-pigmented Intracellular Bodies.**—These apparently represent the earliest stage in the development of the parasite, and are found in all forms of malarial fever; they are especially abundant during the latter part of the paroxysm or immediately thereafter. In the wet specimen they may at first sight be mistaken for vacuoles, but upon closer examination it will be found that they exhibit distinct movements of an ameboid character, and may thus easily be recognized with a little experience. The rapidity with which these changes in form occur in the tertian type of ague is most astonishing, and sketches of any one phase can often, indeed, be made only from memory; in quartan fever the movements are much slower and far less extensive. In the irregular fever of the estivo-autumnal form ameboid movements may likewise be observed, but more commonly the parasite assumes a ring-like appearance, and does not throw out distinct pseudopodia. If these forms are carefully observed, however, it will be found that they are not absolutely quiescent, but alternately expand and contract.

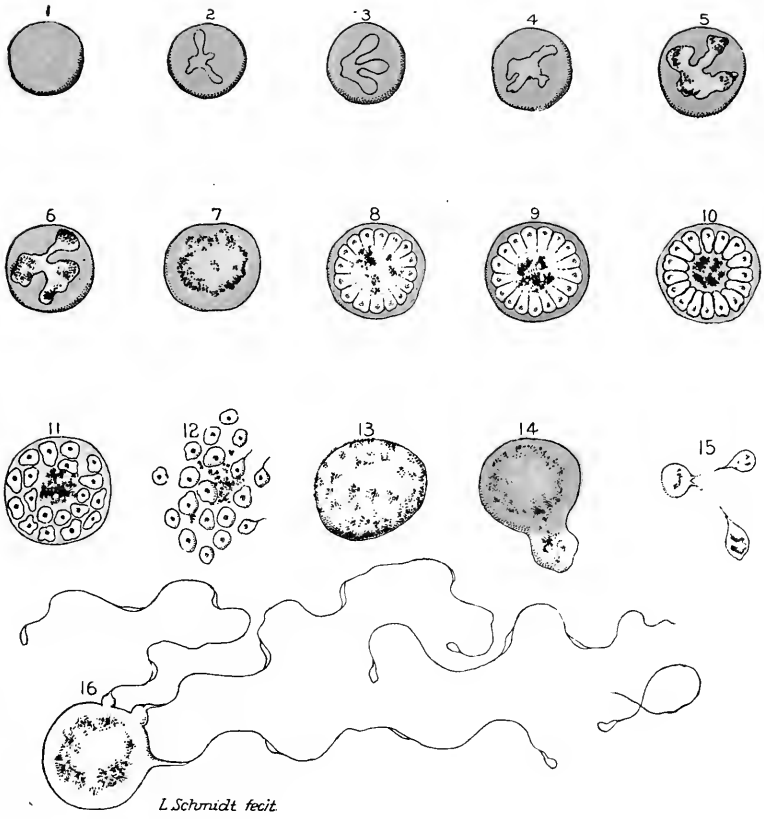
[1] This is an aqueous solution of borax (5 per cent.) and methylene blue (2 per cent.). The blood films are stained with this solution for about thirty seconds; they are then washed in water, dried with filter paper, and afterward by gently warming them over the flame.

In tertian fever the organism (Plate X) is pale and indistinct, while in quartan fever it is sharply outlined and somewhat refractive (Plate XI, Fig. 2). In the estivo-autumnal form the organism is usually much smaller than in the tertian type, and the ring-like bodies frequently present at some point in their interior a distinctly shaded aspect which closely resembles the darker portion in the centre of a normal corpuscle (Plate XI, Fig. 1). In the stained specimen it is impossible to distinguish between the tertian and quartan parasites at this stage of their development. Both appear as little rings containing a tiny granule of chromatin set in the periphery. The estivo-autumnal organism also is sometimes indistinguishable from the others but occasionally its chromatin is more rod-like in appearance.

2. **Pigmented Intracellular Organisms.**—These represent a later stage in the development of the parasite, and, like the non-pigmented intracellular bodies, are met with in all types of malarial fever. Their appearance, however, differs considerably in the various forms. In tertian fever minute granules of a reddish-brown color appear in the bodies of the organism soon after the paroxysm. These gradually increase in number, while the invaded corpuscles proportionately become paler and paler, until finally only an indistinct, shell-like outline can be discerned. In fresh specimens the granules, which often assume the form of little rods, resembling bacteria, exhibit most active molecular movements, attracting attention at once. The body of the parasite, which during its development has increased gradually in size, is probably hyaline, and may still be seen to undergo ameboid movements. These are not nearly so active, however, as in the non-pigmented stage. The movements, moreover, cannot be followed so readily, owing to the presence of the granules. At first sight these appear to be scattered in small collections throughout the red corpuscle, and the impression may be gained that several organisms are present in the same cell. Upon closer investigation, however, it will be seen that this is only apparently the case, and that the granules are confined to the bulbous extremities of the pseudopodia of a single parasite. Before the end of forty-eight hours the organism has filled out the entire red corpuscle, which at the same time has attained a larger size than before. The ameboid movements become less and less marked, and the pigment granules, which may still be quite active, tend to collect about the periphery (Plate X).

In quartan fever pigmented intracellular bodies likewise appear soon after the paroxysm. The individual granules, however, are somewhat larger, of more irregular size, and darker in color than those seen in the tertian type (Plate XI, Fig. 2). Instead of exhibiting active molecular movements, moreover, they are almost entirely quiescent, and usually are grouped along the periphery of the organism. While ameboid movements can at first be observed, they become less and less marked, until finally, at the end of from

PLATE X



The Parasite of Tertian Fever.

1, normal red corpuscle; 2 to 4, non-pigmented stage of the organism, showing ameboid movements; 5 to 7, progressive pigmentation and growth; 8 to 11, process of segmentation; 12, young forms; 13, large extracellular organism; 14, mode of formation of extracellular body; 15, small fragmented extracellular organism; 16, flagellated body and free flagella. Unstained specimen. (Personal observation.)

sixty-four to seventy-two hours, they cease. The organism then presents a round or ovoid form, but does not fill the red corpuscle entirely. It is curious to note that in this form of ague the red corpuscles do not become decolorized, but rather darker than normally, and at times specimens may be seen which present a distinctly greenish or brassy appearance. When the parasite has become fully developed the corpuscle is smaller than normally, and, on staining, it may be seen that the organism still is surrounded by a narrow zone of corpuscular protoplasm even when this is not apparent in unstained preparations.

The pigmented intracellular bodies which may be found in estivo-autumnal fever (Plate XI, Fig. 1) can readily be distinguished from those observed in tertian and quartan ague. As in these types, pigment granules also appear after the paroxysm; they are never numerous, however, and often only one or two minute dark granules can be detected near the periphery. The organism, even in the later stages of its development, scarcely ever occupies much more than one-third of the corpuscle. Usually the granules exhibit scarcely any movements. As in the quartan type of ague, decolorization of the red corpuscles does not occur, and here, as there, a greenish, brassy appearance often is observed.

At the beginning of and during the paroxysm forms are at times seen in which the few pigment granules that may be present have gathered in the centre of the parasite and formed a solid clump. From the fact that these are observed only during the paroxysm, and that central blocks of pigment are found only during the stage of segmentation (see below) in tertian and quartan ague, Thayer and others conclude that these bodies are presegmenting forms of the parasite. This belief is strengthened by the observation that pigment-bearing leukocytes are then also seen, which in the other types of fever likewise are found only at this time.

3. **Segmenting Bodies.**—In cases of tertian and quartan fever the process of segmentation may be observed directly under the microscope, if specimens of blood are obtained just prior to or during the chill. In tertian fever organisms will then be seen in which the destruction of the red corpuscles has advanced to a stage at which it is only possible to make out a pale contour of the original host. The parasite itself has gradually assumed a granular appearance, and the pigment granules, which until then have exhibited pronounced molecular movements, now become quiescent, larger and rounder, and show a distinct tendency to collect in the centre of the body. Here they form a roundish mass in which the individual components can scarcely be made out. While this change in the position of the pigment is taking place, beginning segmentation of the surrounding granular protoplasm will be observed. This at first is most marked at the periphery, from which delicate striæ will gradually be seen to

extend toward the central mass, dividing up the protoplasm into a number of oval bodies, giving rise to appearances which resemble the petals of a flower (Plate X). Still later these bodies, which in reality are the sporules (merozoites) of the parasite, will be found scattered in an irregular manner throughout the interior of the organism. The apparent envelope then disappears, and the sporules, which in tertian fever usually number from fifteen to twenty, lie free in the blood. Quite frequently, also, a sudden expulsion of the little bodies is observed and the impression gained as though the envelope had been burst asunder. Upon closer inspection, even at the petal stage, it will be seen that almost every sporule presents a tiny dot in its interior, which may at first sight be mistaken for a pigment granule, but which in all probability is composed of nuclear material. After the expulsion of the sporules these are frequently seen to move about in an active manner, but sooner or later they come to rest.

While the progress of segmentation is usually observed to proceed in the manner described, this is not invariably the case. It may thus happen that segmentation occurs before the pigment granules have had time to gather at the centre, or that the parasitic protoplasm breaks up into sporules directly without the intervention of the petal stage. In every case, however, the formation of sporules is associated directly with the occurrence of a paroxysm and represents the asexual type of reproduction of the parasite (schizogony).

The sporules, unless destroyed by leukocytes, in turn invade new corpuscles, cause their destruction, and become segmented, thus giving rise to a new generation. As the process of segmentation coincides in time with the occurrence of the chill, it is apparent that the interval elapsing between two consecutive chills—*i. e.*, the type of the ague—depends upon the rapidity with which the organisms arrive at maturity.

A distinction between the benign tertian parasite (plasmodium vivax) and the pernicious type (P. falciparum) is not always possible. The latter during segmentation may produce as many as 32 spores, while the former usually forms 16. In P. vivax the pigment usually remains scattered throughout the body of the parasite until just before segmentation, while in P. falciparum the condensation begins already quite early during its growth.

In quartan ague segmentation differs somewhat from that observed in the tertian form. It will here be observed that the pigment granules, which have gathered along the periphery of the organism, as the parasite approaches maturity become arranged in a stellate manner, and apparently reach the centre through definite protoplasmic channels. Here they form a dense clump, and while the protoplasm assumes a finely granular appearance, segmentation proper begins and proceeds as in the tertian form. The number of segments, however, is smaller, varying between six and fourteen (average of

eight). The entire segmenting body, moreover, is smaller than in the tertain form, and the segments are arranged in a more symmetrical manner. Here, indeed, the most perfect rosettes are observed (Plate XI, Fig. 2).

In estivo-autumnal fever segmenting bodies are only exceptionally seen in the peripheral blood, and it appears that the process of reproduction occurs principally in the spleen. The segments, as a rule, number from eight to twenty-five (average of twelve). The segmenting body itself, however, is much smaller than in either the tertian or quartan form, and it is not possible to distinguish any remains of the original host.

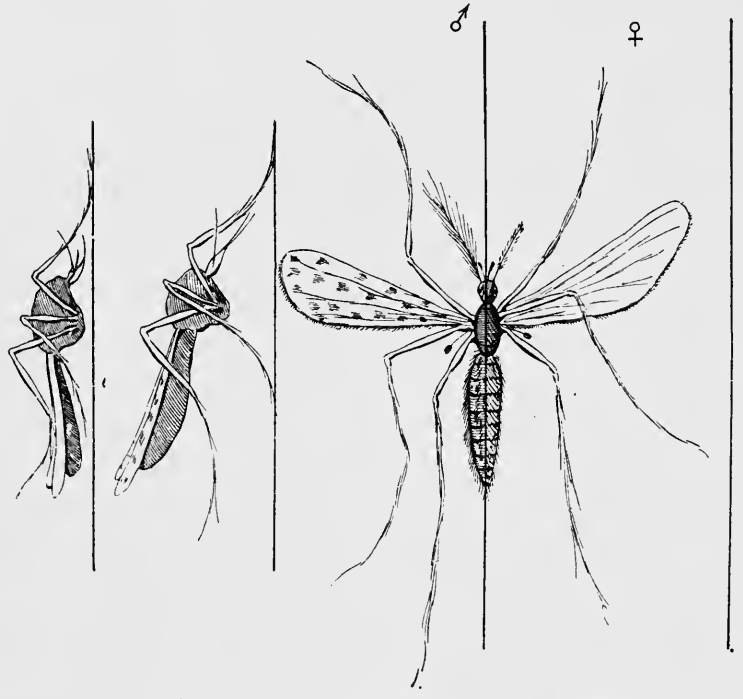


FIG. 40.—(From Döflein.)

4. **Extracellular Pigmented Bodies or Gametes.** — In tertian and quartan ague some of the pigmented intracellular bodies, instead of undergoing segmentation when they have arrived at maturity leave their hosts and appear as such in the blood. Some of them at the same time increase considerably in size, and in the tertian form may become as large as a polynuclear leukocyte (Plate X). The pigment granules, moreover, exhibit an activity in their move-

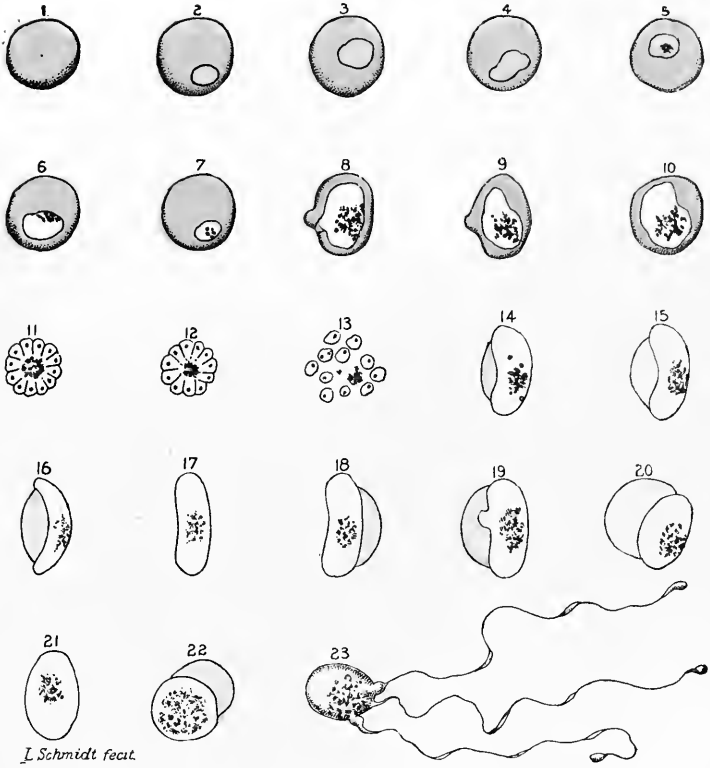
ments which is most astonishing and never observed under ordinary conditions. Upon careful observation it will be seen that in some of the bodies the movements of the granules after a while become less and less marked, and finally cease, while the body of the parasite itself becomes irregular in outline. This appearance is undoubtedly referable to the death of the organism. In others a gradual fragmentation is observed, small particles of the pigmented mother-substance being cut off from the parent form. It is thus quite common to see the original parasite break up into four or five smaller bodies, in which the movements of the pigment granules persist for some time. Sooner or later, however, even these cease, the outlines of the bodies become more and more indistinct, and death occurs. In still others the formation of vacuoles may be observed, the pigment granules at the same time becoming quiescent. This process is likewise regarded as one of degeneration. Most interesting, however, is the fact that *flagellation* may occur in some of these extracellular forms. This may sometimes be hastened in the wet specimen by gently breathing upon the slide so as to form a thin film of moisture. It will then be observed that the pigment granules which exhibit a most surprising activity tend to collect near the centre of the organism, while at the same time curious undulating movements may be made out along its contours. Suddenly one or more (one to six) slender filaments will be seen to protrude from as many points on the periphery, presenting minute enlargements here and there in their course (Plate XI) (polymites). The length of these filaments, or flagella, as they have been erroneously termed, varies considerably. As a rule, it does not exceed the diameter of from five to eight red corpuscles. With these flagella the organism makes most active whipping movements, scattering the red corpuscles to the right and left. Attention is, indeed, usually drawn to the presence of these bodies by the disturbance which they cause in the field of vision. Occasionally one of the flagella may be seen to become detached from the body of the parasite and to move rapidly about among the corpuscles in a snake-like manner. In microscopic specimens they gradually come to a rest and often curl into a spiral.

Beyond the fact that the flagellate organisms in tertian fever are larger than in the quartan form, no special points of difference exist (Plate XI, Fig. 2).

In estivo-autumnal fever similar changes may be observed. The appearance of the flagellate bodies, however, is here preceded by the development of crescentic forms, which themselves become ovoid in shape and then spheroid. Some of the spheroids then become flagellated. These extracellular types are observed in cases of estivo-autumnal fever, after the disease has persisted for at least a week. At first sight they bear no apparent relation to the intracellular forms, but it has been definitely ascertained that they develop from these. Speci-

PLATE XI

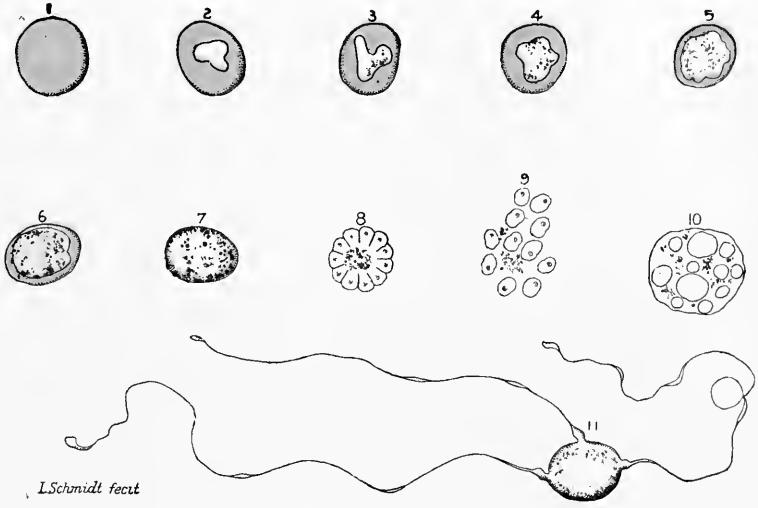
FIG. 1



The Parasite of Estivo-autumnal Fever.

1, normal red corpuscle; 2 to 10, gradual growth of the organism; 11 and 12, segmenting bodies; 13, young forms; 14 to 22, crescents, ovoids, and spherical bodies, with and without bib; 23, flagellated body. Unstained specimen. (Personal observation.)

FIG. 2



The Parasite of Quartan Fever.

1, normal red corpuscle; 2 to 6, gradual growth of the organism; 7, pigmented extracellular body; 8, segmenting body; 9, young forms; 10, vacuolated extracellular body; 11, flagellated form. Unstained specimen. (Personal observation.)

mens may, indeed, be met with in which the crescentic bodies are seen in the interior of red cells which have lost but little of their original color. But this is not common. The typical extracellular crescents are fairly refractive little bodies, which are somewhat larger than a red cell measuring about 7μ to 9μ in length by 2μ in breadth. Their extremities are usually rounded off and joined by a delicate curved line which represents the remains of the original host;

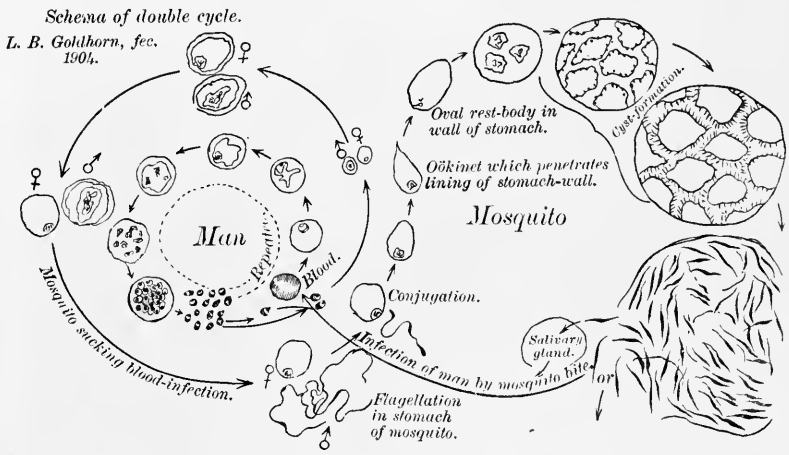

Schema of double cycle.
L. B. Goldhorn, fec.
1904.
Oval rest-body in wall of stomach.
Cyst-formation.
Oökinet which penetrates lining of stomach-wall.
Mosquito
Man
Gametes
Blood.
Conjugation.
Mosquito sucking blood-infection.
Infection of man by mosquito bite
Salivary gland.
or
Flagellation in stomach of mosquito.
Inner circle-asexual reproduction; moist-chamber observation shows no flagellation. Outer circle-formation of (sexual) gametes; moist-chamber observation shows flagellation.
Infection of man through gastro-intestinal and respiratory tract, the infected mosquito dying in water, drying in air or sucking plant-juices, infecting fresh vegetables (theoretical)

FIG. 41.—Illustrating cycle of development. (Park.)

FIG. 42.—Oökinetes of pernicious parasites in the stomach of Anopheles maculipennis thirty-two hours after having been sucked in. (Grassi.)

at other times the little bib is seen on the convex border. The little pigment granules which are always found in the interior are generally collected about the centre of the body, but may migrate into the horns. The estivo-autumnal flagellates, as in quartan fever, are smaller than those observed in the tertian form (Plate XI, Fig. 1).

The significance of the flagellate organisms is now well under-

stood. They represent the male element in the sexual reproduction of the malarial parasite (microgametocytes) and the beginning of a cycle of development, which takes place outside of the human body, in the bodies of mosquitoes of the species Anopheles. The beginning of this cycle was first observed by MacCallum in the blood of infected crows. He here discovered that when one of the flagella (micro-

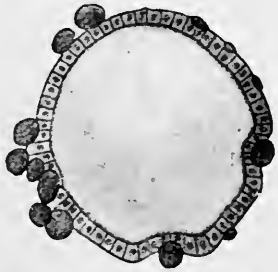


Fig. 43.—Transverse section of the stomach of an anopheles, with cysts of pernicious parasites. (Grassi.)

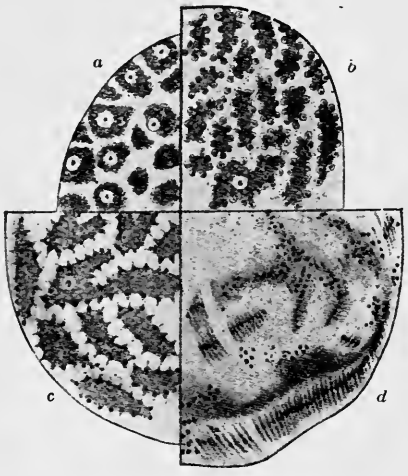


Fig. 44.—Four stages of sporulation of malarial parasites from Anopheles maculipennis strongly magnified: *a-c*, the estivo-autumnal parasite; *a*, four to four and a half days after ingestion; *b* and *c*, five to six days after ingestion; *d*, tertian parasite, eight days after ingestion (Grassi.)

gametes) broke loose it almost always sought out another full-grown form of the parasite which had not undergone segmentation, and penetrated this, just as the spermatozoön penetrates the ovum. Subsequently he observed the same process in the blood of the human being, which has since been confirmed by others. The female cells

are somewhat larger than the male cells and termed macrogametes. The further development (sporulation) of the fertilized forms, oökinetes (Fig. 42), does not take place in the human being, but in mosquitoes. The fertilized organism penetrates the stomach wall of the insect and here gives rise to the formation of little cysts (oöcysts) (Fig. 43), in which after about seven days numerous irregular, rounded, ray-like striæ appear (Fig. 44). After a time the capsules of the cysts burst and the delicate, thread-like bodies (the sporozoites) are set free in the body cavity of the mosquito, and shortly after appear in the salivary glands (Fig. 45). These bodies represent the young parasites, which result from the sexual reproduction of the adult organism.

FIG. 45.—Section through a tubule of the salivary gland of an anopheles, with sporozoite. of the estivo-autumnal parasites; above an isolated sporozoite with higher magnifications (Grassi.)

If at this stage of their development the infected mosquito is allowed to bite a human being malarial infection results, with the appearance in the blood of the hyaline forms already described.

From the above description it will be seen that three forms of the malarial parasite may be found in the blood, viz., the parasite of tertian, quartan, and estivo-autumnal fever, and it has been shown that these forms may be distinguished from each other. In tertian and quartan fever several groups of the same organism may be present at one time, and as the process of segmentation coincides with the occurrence of a paroxysm it will readily be seen that the number of paroxysms within a given time depends upon the number of groups

which may be present in the blood. If a double infection with the tertian parasite has occurred, one group of organisms may just have reached the segmenting stage, while the second group has attained only a twenty-four hours' growth, the result being that maturity is reached by the two groups on successive days. Quotidian fever is the result. In quartan ague, similarly, double quartan fever will occur if two groups are present, and triple quartan fever if three groups are present at one time. Should still other groups be present, the clinical picture will accordingly become more complicated. Mixed infections, further, are also possible.

Pigmented Leukocytes.—In conclusion, it may not be out of place to refer to the presence of pigment-bearing leukocytes in the blood of malarial patients (Plate XII). These are quite constantly met with during the paroxysm, and it is indeed often possible to observe the process of *phagocytosis* directly under the microscope. The forms which are taken up are the small, fragmented, extracellular forms, the flagellate bodies, segmenting bodies, and free pigment clumps. In every case where pigment-bearing leukocytes are observed, malarial fever should be suspected and a careful examination made, as a melanemia occurs only in this disease, in relapsing fever, and in connection with melanotic tumors, in which not only leukocytes containing melanin may occur in large numbers, but also masses of pigment floating free in the blood.

TRYPANOSOMIASIS

The first authentic report concerning the occurrence of trypanosomiasis in man was made by Dutton in 1902, while in animals their occasional presence had long been recognized (frogs, rats, dogs, groundhogs, etc.). In tropical regions certain species are pathogenic for certain domestic animals. The tsetse fly disease or Nagana of Africa, the Surra disease of Asia, and the mal de caderas of South America are all referable to infection with trypanosomes (observed in the horse, the African buffalo, the ox, the donkey, mule, antelope, camels, and elephants). Especially interesting is the observation of Castellani and Bruce of the association of trypanosomiasis with a certain symptom complex, of which the so-called sleeping sickness is one of the possible manifestations. Bruce could demonstrate the organism in the blood of 12 out of 13 cases, and in the cerebrospinal fluid in all of 38 cases. The findings of these earlier observers have since been abundantly confirmed, and it is now generally conceded that the disease in question is referable to infection with trypanosomes.

The Trypanosoma gambiense (Dutton) is from 8 to 25μ long and from 2 to 2.8μ broad. It is provided with an undulating mem-

PLATE XII

Malarial Organisms

brane and a flagellum, which starts from a centrosome or micronucleus lying in the posterior end of the animal, and projects somewhat

FIG. 46.—Trypanosoma gambiense (sleeping sickness) in blood of a rat. Two types are shown; the broad, pale form (female?) is dividing. Magnification 1500 times. MacNeal's stain. (From Novy.)

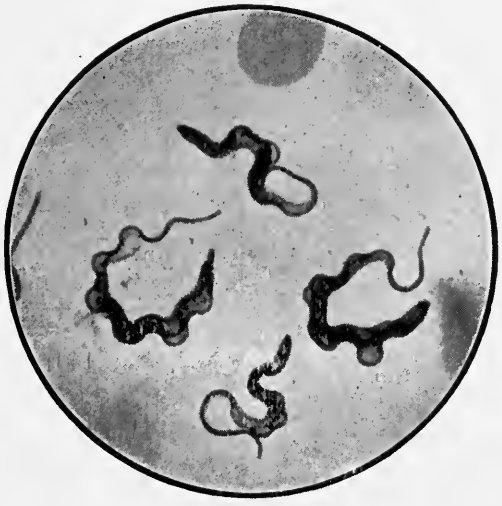


FIG. 47.—Trypanosoma gambiense from same preparation as preceding, showing the usual form; some cells in process of division. Magnification 1500 times. (From Novy.)

beyond the anterior end (Figs. 46 and 47). There is an oval nucleus which is centrally located and is made up of chromatin granules.

In the wet preparation the organism exhibits slow spiral movements. It is found free in the blood plasma, but may also be seen in the interior of leukocytes, which latter manifestly destroy the organisms exactly as the malarial parasites. In dry specimens the trypanosomes can be stained with any basic dye; with the Romanowsky stain or one of its modifications it is stained like the malarial organism. Levaditi recommends the following method as especially valuable: Fixation in absolute alcohol and ether for five minutes; primary staining for two minutes with a saturated solution of Bismarck brown, followed by washing and counterstaining with Unna's polychrome blue (diluted one-half with water) for two minutes. The specimens are rinsed in water, dried carefully over a flame, and examined as usual. Wilson's stain, Hastings' stain or Giemsa's may be used in place of that of Unna.

The number of organisms in a blood preparation is rarely large; as a rule, not more than from 3 to 8 are found to a cover-slip. During apyrexia they are not seen.

Infection in man occurs through a biting fly—the Glossina palpalis, which supposedly transmits the disease in a purely mechanical way.

Novy and McNeal have succeeded in cultivating the trypanosoma of Bruce in the water of condensation from a medium of agar mixed with defibrinated rabbit's blood (1 to 1) at 25° C., and the rat trypanosome (Trypanosoma lewisi) in a similar medium containing 1 part of blood for 2, 5, or even 10 parts of agar.

RELAPSING FEVER

Relapsing fever is characterized by the presence in the blood, and here only, of spirochetes which bear the name of their discoverer, Obermeier. In order to search for the organisms no special precautions are necessary. After having cleansed the finger a drop of blood is mounted on a thin cover-glass, which is inverted upon a slide and is then ready for examination; an oil-immersion lens is not a necessity, but preferable to the middle power. Attention is drawn to the presence of the organisms by disturbances which are noticeable among the red corpuscles, and upon careful focussing it will be seen that these are caused by the wriggling movements of the spirochetes. The Spirochætæ Obermeieri are long, slender filaments, measuring from 36μ to 40μ in length by 0.3μ to 0.5μ in breadth, and present from eight to twelve convolutions of equal size, with tapering extremities. These two last characteristics serve to distinguish this species from that described by Ehrenberg, in which the radius of the incurvations is not the same in all, and in which the extremities do not taper (Fig. 48).

Culture experiments have not been very satisfactory, although

Koch observed an increase in their number at a temperature of from 10° to 11° C.

Koch has shown that in African relapsing fever, which is likewise due to a spirochete, infection occurs through the bite of a certain tick, Ornithodorus moubata, which acts as intermediary host in the development of the organism, the ovaries being the organ in which this takes place.

The tick fever of the Congo Free State is apparently identical with the African recurrens described by Koch. Infection likewise occurs through the bite of infected ticks, Ornithodorus moubata. The same is probably true of the relapsing fever of China. In a specimen of blood from such a patient, which I owe to the kindness of Dr. Logan of the Chinese mission, spirochetes were present in large numbers.

Hödlmoser has shown that the blood of recurrens is spirilla agglutinating. But as the culture of the organisms is practically not possible, the blood of a second case must be available for the test.

FIG. 48.—Spirochæte Obermeieri; blood smear. × 1000 diam. (From Itzerott and Niemann.)

TROPICAL SPLENOMEGALY (KALA-AZAR)

Through the researches of Donovan, Leishman, and Ross especially it has been established that in tropical splenomegaly (cachexial fever, Kala-azar) parasites may be demonstrated in the blood which are probably the causative factor of the disease in question. The organism has been termed the Leishmania Donovani (Leishman-Donovan body, Cunningham-Leishman-Donovan body). It represents a stage in the development of a trypanosome, as was first suggested by Rogers and as has since been shown by cultural experiments by Leishman and Statham.

In the *peripheral* blood the organisms are rarely found and only when the temperature is high. Splenic puncture gives the best results.

Donovan suggests that it is well to keep the patient flat on the back for twenty-four hours after the operation and to give a dose of cal-

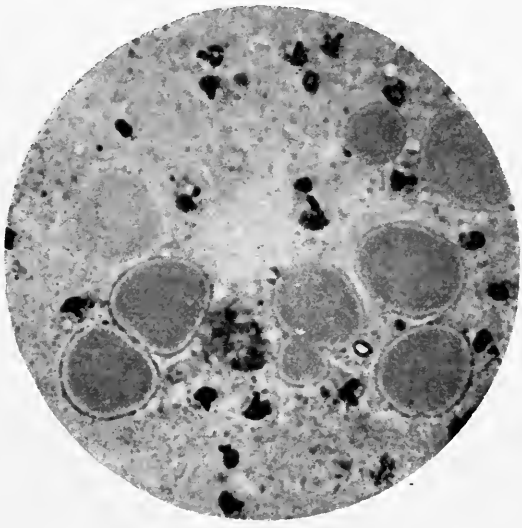


FIG. 49.—Leishmania donovani. In splenic smear from a case of kala-azar. Wright's stain × 1800. (From Bull. 1, 1913, Surgeon-General's Office.)

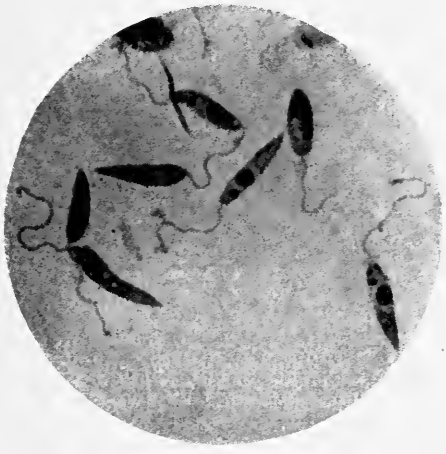


FIG. 50.—Leishmania donovani. Flagellated form from a culture. Wright's stain. × 1800. (From Bull. 1, 1913, Surgeon-General's Office.)

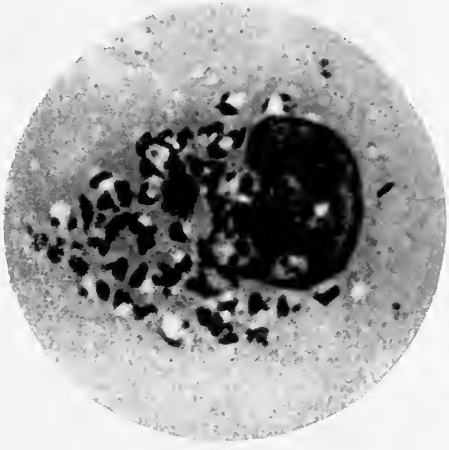
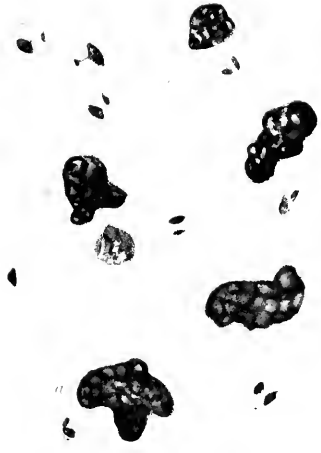


FIG. 51.—Leishmania tropica. In a smear made from a tropical ulcer. Wright's stain. × 1800. (From Bull. 1, 1913, Surgeon-General's Office.)

cium chloride immediately after and twice again at intervals of three hours (to prevent hemorrhage). The parasites are principally met

PLATE XIII



with in large mononuclear cells. The typical forms are oval or circular with a well-marked contour (Plate XIII). There is a deeply staining nucleus lying against the capsule and a deeply staining rod-like centrosome. They may occur singly or in pairs or in zoöglœa masses (Figs. 49 and 50). They are readily stained with any one of the methylene-azure mixtures (Hastings, Giemsa, Leishman, etc.).

SYPHILIS

The Spirochæte pallida (Treponema pallidum) has been demonstrated in the blood during life. Under ordinary circumstances, however, its search is here not likely to be attended by success. For diagnostic purposes it should be looked for in scrapings from chancres, papules, condylomas, in the aspirated juice of enlarged lymph glands, etc. (For a description of the organism, see Examination of Syphilitic Material.)

SPOTTED FEVER

In the so-called spotted fever, which occurs in Montana, Nevada, Oregon, etc., an intracorpuscular ameboid, non-pigmented organism has been described by Wilson and Chowning, as also by Anderson, which they regard as the cause of the disease. They term this the Piroplasma hominis. Infection supposedly takes place through ticks belonging to the species Dermacentor reticulatus.

I have studied the blood from several cases which were placed at my disposal by Drs. McCalla, Maxey, Pease, and Parsons, but was unable to find such structures. Craig and Stiles express themselves in a similar manner.

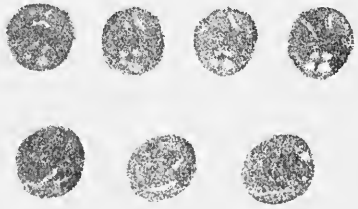


Fig. 52.—Parasite of oroya fever in fresh blood specimen (baeillary and rounded forms).

OROYA FEVER

Oroya fever has been shown to be due to infection with a protozoan parasite related to the group of lymphohematocytozoa and termed *Bartonella bacilliformis* (Harvard expedition to South America, 1913). The organism is found within the red cells and in stained preparations (Giemsa) appears either as a rod-shaped body, measuring about 1 to 2μ in length by 0.2 to 0.5μ in breadth, or as a rounded

form with a diameter of from 0.3 to 1μ (see Figs. 52 and 53). The number of organisms found in a red cell may vary from one to ten, the severity of the case depending upon the number of cells infected.

The parasite may also be seen in the unstained preparation, but is then frequently difficult to detect. It apparently is capable of changing its position within the cell.

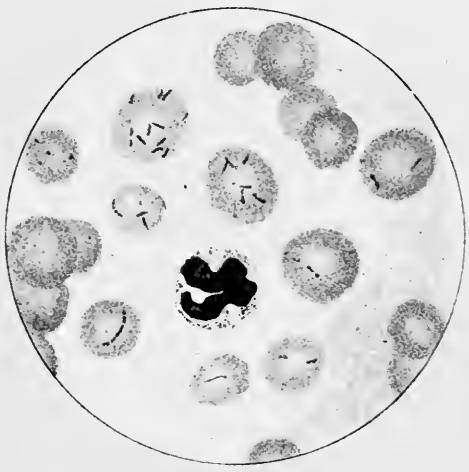


Fig. 53.—Parasite of oroya fever in stained blood specimen.

FILARIASIS

According to Manson, the embryos of at least four, and possibly five and even more distinct species of nematodes may be found in the blood of man. These various blood worms Manson designates as the Filaria nocturna, Filaria diurna, Filaria perstans, Filaria demarquaii, Filaria ozzardi (a doubtful species), and a sixth, which may or may not be connected with one of the two last, the Filaria magelhæsi. Two of these at least are of pathological import, viz., the Filaria nocturna and the Filaria perstans.

Filaria Nocturna (Manson): *syn.*, Filaria sanguinis hominis (Lewis). This filaria is the embryo form of the Filaria Bancrofti (Cobbold), which inhabits the lymphatics and is unquestionably the cause of endemic chyluria, of various forms of lymphatic varix, of tropical elephantiasis arabum, and possibly also of other obscure tropical diseases. The organism in question is widely distributed. It is indigenous in almost all tropical and subtropical countries as far north as Spain in Europe and Charleston in the United States, and as far south as Brisbane in Australia. It is very common in

Cochin and in some of the South Sea Islands, where one-third and one-half of the population, respectively, appear to be infected.

In the following description of both parent and embryo form I quote largely from Manson's account of the parasite in his admirable *Lectures on Tropical Diseases.*

The parent filarias are hair-like, transparent worms measuring from 7.5 to 10 cm. in length. The sexes live together, often inextricably coiled about each other. Sometimes they are inclosed, coiled several in a bunch, and tightly packed in little cyst-like dilatations of the distal lymphatics; sometimes they lie more loosely in lymphatic varices; sometimes they inhabit the large lymphatic trunks between the glands, the glands themselves, and probably not infrequently the thoracic duct. The female is the larger; there are two uterine tubes

FIG. 54.—Filaria sanguinis.

which occupy the greater part of the body, and which are filled with ova in various stages of development. The vagina opens near the mouth; the anus just in advance of the tip of the tail. The cuticle is smooth and without markings. In both sexes the mouth end tapers slightly; it is clubbed and simple. The male is characterized by its marked disposition to curve. The cloaca gives exit to two slender, unequal spicules

In the wet preparations the Filaria nocturna appears as a transparent, colorless little worm, which wriggles about most actively, constantly agitating and displacing the corpuscles in its vicinity. It will be noticed, however, that the animal does not propel itself through the drop of blood, but remains stationary. At first the movements are so active that it is impossible to make out any anatomical details; after a number of hours, however, the movements become more sluggish, and it is then possible to study the worm with more ease. It measures about 0.31 mm. in length by 0.007 to 0.008 mm. in width.

With the higher power it will be seen that the entire worm is inclosed in a delicate envelope, in which it moves backward and forward, the sheath being much larger than the worm (Fig. 54). It is owing to the presence of this sheath that active locomotion on the part of the worm is not possible. About the posterior part of the middle third of the parasite there is an irregular aggregation of granular matter, which represents a viscus of some sort. With a high power one can further make out a delicate transverse striation in the musculocutaneous layer throughout the entire length of the animal. In stained specimens two V-shaped light spots can be made out: one at a point about one-fifth of the entire length of the organism, backward from the head end; the other, very much smaller, a short distance from the tail. The first Manson designates the "V" spot, the second the tail spot. In stained specimens these two spots are readily made out, as they do not take the color. When the movements of the animal have almost ceased, one can see on careful focussing that the head is constantly being covered and uncovered by a six-lipped or hooked and very delicate prepuce; and, moreover, one can sometimes see a short fang of extreme tenuity suddenly shoot out from the uncovered extreme cephalic end and as suddenly retracted.

Technique.—The examination should be made late in the evening, after the patient has rested for a number of hours. Drops of blood are then mounted, wet, on slides and ringed with vaselin to prevent the specimen from drying. In such preparations the filarias keep alive for a week or longer. They should be searched for with a low power—an inch objective is very convenient for the purpose. Attention is directed to their presence by the commotion which they cause among the neighboring blood corpuscles.

To prepare permanent mounts, blood smears are best made on slides, which are then stained with eosinate of methylene blue in the usual manner. Working with the blood of infected animals, I have thus obtained very good results. The V and tail spots are very well brought out. To show anatomical details, however, staining with eosin and hematoxylin, after fixing the smears with alcohol, gives the best results; in this manner the sheath is very well shown, as also the structure of the musculocutaneous layer.

Infection occurs through the females of mosquitoes belonging to both the culex and anopheles family which have fed on the blood of filaria-infected individuals. The history of the parasite while in the body of the mosquito is in brief the following: After their arrival in the stomach the young worms shed the sheath and invade the thoracic muscles, where they increase in size (to 1.5 mm.), develop a mouth, an alimentary canal, and a trilobed tail. They then find their way into the abdomen, where, in suitably prepared sections, they may occasionally be seen in the tissues about the stomach, and even among the eggs in the posterior part of the abdomen. The majority now

find their way to the base of the proboscis and under appropriate conditions out through the proboscis by a channel which they make for themselves. After introduction into the human body the organism finds its way into the lymphatics, where it attains sexual maturity; fecundation takes place and the new generation of filarias enter the blood current by way of the thoracic duct and the left subclavian vein. The development of the embryo form in the mosquito occupies from sixteen to twenty days.

Whether or not infection can occur in any other way is not known. We could conceive that some of the worms are eliminated with the eggs of the mosquitoes, and that infection could then take place through contaminated drinking water.

Filaria Perstans.—This species is of interest, as it was thought to be concerned in the causation of the so-called sleeping sickness of tropical Africa which now, however, is known to be due to infection with trypanosomes. It has likewise been found in the Buck Indians of British Guiana, among whom the same sickness occurs. The organism observes no periodicity, but is present in the blood both during the daytime and at night.

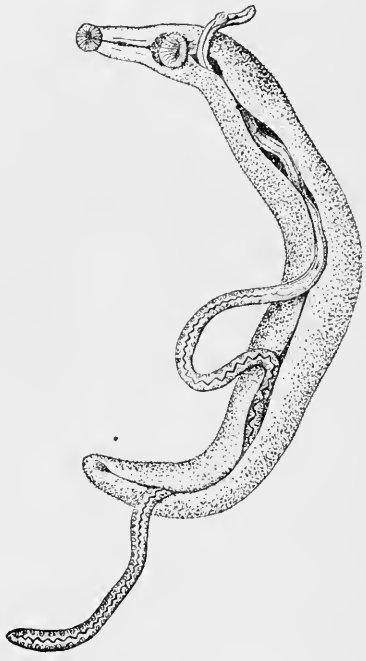


Fig. 55.—Male and female specimens of the human blood fluke (Bilharzia hæmatobia). × 12. (After Looss.)

The embryo worm is smaller than the Filaria nocturna; it measures about 0.2 mm. in length by 0.004 mm. in breadth. It has no sheath, and its caudal end is truncated and abruptly rounded. There is no hooked cephalic prepuce. Its motion is progressive.

The adult form measures 70 to 80 mm. in length. The tail in both sexes is incurvated and the chitinous covering at the extreme tip split, as it were, into two minute triangular appendages. They have been found in the connective tissue, at the root of the mesentery, behind the abdominal aorta, and beneath the pericardium.

DISTOMIASIS (BILHARZIASIS)

Bilharzia hæmatobia (Cobbold): *syn.*, Gynæcophorus (Diesing) Distomum hæmatobium (Bilharz); Schistosoma hæmatobium (Weinland); Distoma capense (Harley); Thecosoma (Maguin-Tandon)

The Bilharzia hæmatobia belongs to the class of trematode platodes. According to Bilharz, the greater portion of the Fellah and Coptic population of Egypt is infected. It is common in South Africa, and has also been observed in Mesopotamia, and apparently in Arabia. In the United States a few isolated cases have been seen which were undoubtedly imported. From Europe no endemic cases have been reported. The parasite may give rise to diarrhea, hematuria, and ulceration of the mucous surfaces.

The male is smaller but thicker than the female, measuring from 12 to 15 mm. in length by 1 mm. in breadth. On its abdominal surface a deep groove is found with overlapping edges, which serves for the reception of the female (Fig. 55). It has an oral and a ventral sucker placed close together.

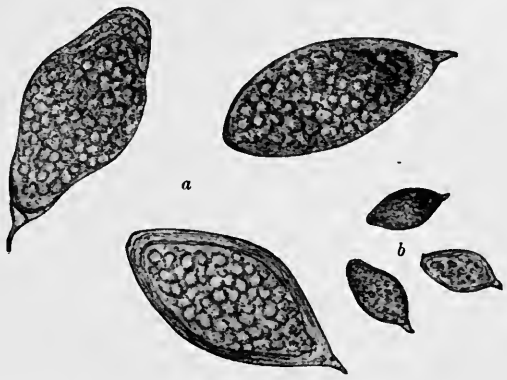


FIG. 56.—Bilharzia eggs from the urine: Group *a* was drawn to scale with B. & L. $\frac{1}{6}$ obj. and 1 in. ocular; group *b* represents their appearance with B. & L. $\frac{2}{3}$ obj.

The adult parasites are found in the blood of the portal vein, in its mesenteric and splenic branches, and in the vesical, uterine, and hemorrhoidal veins; they have also been found in the vena cava and may possibly occur elsewhere in the circulation. The eggs are more often seen. They are oval bodies, measuring 0.16 mm. in length by 0.05 mm. in breadth, and are provided with a distinct, spike-like projection which issues from one extremity or the side (Fig. 56). Infection usually takes place through unfiltered drinking water, but may also occur through the skin. Through the portal system the parasite invades the urogenital system, the anus, and rectum, and may also proliferate abundantly in the intestine, the liver kidneys, etc. The diagnosis is usually made by examination of the urine, in which the ova will be found.

Another variety of blood fluke has been described by J. Catto, *Schistosoma cattoi;* it was found in a Chinese who had died of cholera

ANGUILLULIASIS

In 1895 Teissier reported a case of intermittent fever in which numerous embryos of anguillula were found in the blood. They disappeared after expulsion of the parasites from the intestinal tract, and at the same time the fever ceased. It is a question, however, whether Teissier's parasite was identical with the common form described by Bavay, Normand, Grassi and others. Unlike the embryos developing from the eggs of both parasitic and free-living generations, Teissier's form did not present the characteristic double œsophageal enlargement, and he reports, moreover, that in the case of the adult male only one instead of two spicules was noted. This view is strengthened by the observation that after inoculation into frogs the worms developed in the intestinal canal and the lungs into giant forms, which may have been *Ascaris nigrovenosa* (*syn.*, Rhabdonema nigrovenosum).

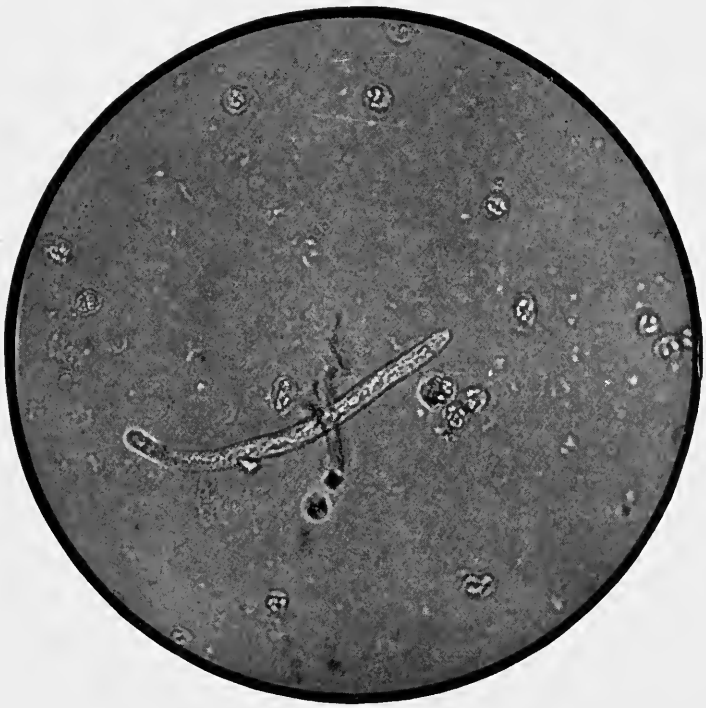


FIG. 57.—Embryo of Trichinella spiralis in blood laked with 3 per cent. acetic acid. Leukocytes and disintegrated red cells are also shown. × 800. (Herrick and Janeway.)

TRICHINOSIS

Through the researches of Staübli, Herrick, and Janeway it has been established that trichina embryos may appear in the blood stream in the corresponding infection and that a positive diagnosis may thus be reached in cases in which the existence of a marked hypereosinophilia has rendered the diagnosis of trichinosis probable. This is exceedingly important, and it is to be hoped that the problem be carefully studied whenever opportunity presents, so that a decision may be reached as soon as possible regarding the frequency of such findings, the stage of the disease at which the embryos appear, the duration of this phase, etc. In Janeway's case the embryos were found on the twenty-third and twenty-fifth day after infection.

TECHNIQUE.—The blood may be obtained either from a vein or the ear; if from the latter source, 2 c.c. should be available; if vein puncture be practicable a larger amount may be procured (5 to 10 c.c.). This is immediately diluted with ten parts of 3 per cent. acetic acid. If the ear is used the drops should be received directly in the acetic acid. The material is then centrifugalized and large drops of the sediment examined with a low power. The accompanying illustration is taken from Janeway. (See Fig. 57.)

THE SEROLOGICAL EXAMINATION OF THE BLOOD

Of the large number of methods which have been devised for the purpose of demonstrating certain reaction products in the blood which appear as a consequence of infection or immunization, in the more general sense of the term (viz., agglutinins, precipitins, cytolysins, antiferments, lipoidophilic antibodies, protective ferments), only those will be considered which have a definite bearing on diagnosis, and are not too intricate for the purposes of the worker in the clinical laboratory.

THE AGGLUTININS

Typhoid Fever.—The Widal Reaction.—The method is based upon the fact that typhoid serum will cause arrest of motility and agglutination of the specific bacilli even when diluted, whereas clumping of the same organism is obtained with sera from other diseases and healthy individuals only when they are used in a more concentrated form. The time limit within which clumping occurs is likewise an important factor, as non-typhoid sera are at times met with in which, notwithstanding a certain degree of dilution, agglutination occurs, providing that the specimen is kept for a long time. Both

factors—viz., the degree of dilution necessary to eliminate the agglutinating power of non-typhoid sera, as also the time limit of observation—have been arbitrarily determined. Widal originally advised a dilution of 1 to 10, and Grüber a time limit of one-half hour. It was soon ascertained, however, that this dilution was too low, and most observers have favored a dilution of 1 to 40 or 1 to 50. At the present time there is a tendency to further increase this even as far as 1 to 200 with a time limit of one-half hour.

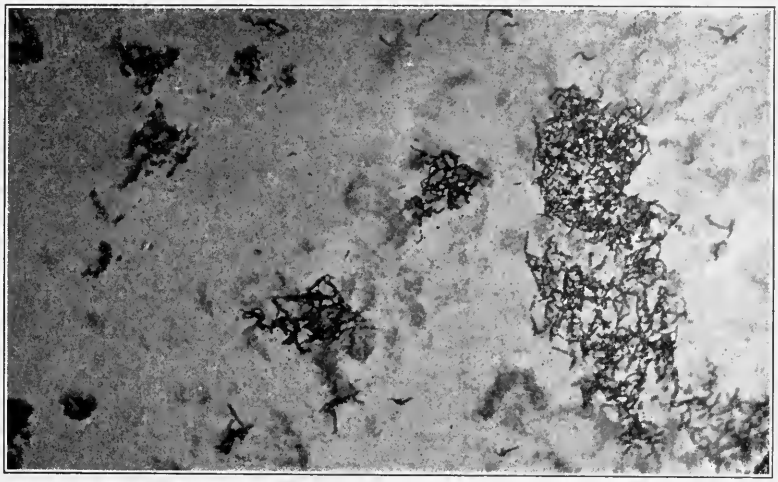


Fig. 58.—Positive agglutinin reaction.

With the original method only a full virulent, fresh bouillon culture of the typhoid bacillus, viz., one not older than sixteen to twenty-four hours, is employed. The further technique is simple: 1 volume of blood serum is diluted with the requisite amount of normal salt solution to 20, 25, 50, or 100 volumes, as the case may be. Of this mixture one droplet is mounted on a cover-glass, mixed with a droplet of the typhoid culture (dilutions of 40, 50, 100, or 200 thus resulting), and inverted over a cupped slide, with a little vaselin along the edges. The examination is conducted with a medium power (Leitz, 6 or 7; Bausch & Lomb, $\frac{1}{6}$).

If the case in question is one of typhoid fever, it will be observed that after a variable length of time the individual bacilli, which at first actively dart about the field of vision, become quiescent, and tend to gather in distinct clumps, while the interspaces become entirely free from bacilli or very nearly so (Fig. 58). After one-half hour, or one or two hours, according to the degree of dilution, all motion has ceased. When the time limit has expired and loss of motility and agglutination

have not occurred the result is reported as negative. In such an event further examinations should be made on the following days. In every case it is well to make a control test with the simple bouillon culture, so as to insure the absence of preformed clumps and the virulence of the organism; of the latter, the degree of motility is the best index. In order to secure the necessary degree of dilution, various methods have been suggested. The simplest, and the one generally employed in municipal bacteriological laboratories, is to receive a large drop of blood upon a slide or slip of glazed paper, and allow it to dry. A drop or two of distilled water is then placed on the blood and allowed to remain for several minutes, when it is further diluted and examined as described. The principal advantage of this method is its simplicity and the fact that the *dried* blood retains its agglutinating properties for weeks and months. The results, however, are less reliable than with the use of liquid blood. This can be readily collected from the ear in plain little glass tubes, or in glass capsules, such as Wright has recommended for opsonic work (Figs. 59–61). The finger or ear is pricked as usual and the blood allowed to enter the bent capillary arm of the capsule by merely being held in contact. When enough has been collected, the far end of the capsule is warmed

Fig. 61

Capillary pipette.

Fig. 60

Wright's blood capsule.

Fig. 59

Tube for collecting blood.

and the straight end sealed, when the blood will mount into the body of the capsule. The bent arm is then also sealed. In this manner the blood can be kept for a long time. At the laboratory it is hung into the centrifuge, if the serum has not already separated out, briefly centrifugated, and the capsule cut with a file. The serum is then diluted with the aid of a Thoma-Zeiss pipette or a common capillary pipette such as anyone can construct and is pictured in Fig. 61. These pipettes are destroyed after use.

A very material advance in the practical application of the agglutination test was made by the discovery that it is not necessary to work with living cultures of the typhoid bacillus, but that dead bacilli will answer just as well, providing they are killed off when in a virulent condition. To this end formalized cultures are especially convenient. To prepare this a twenty-four to forty-eight hours' bouillon culture of an actively agglutinable strain is treated with formalin to the extent of 1 per cent. of the solution, and set aside for a week. The bacteria are allowed to settle, when the supernatant fluid is poured off and replaced by formalized normal salt solution. In this form the material will keep for months. Before use it should be well agitated and examined to see that no artificial clumps are present. With the formalized culture the microscopic examination can then be made, or one can proceed macroscopically. If the microscopic test is used the examination is made after two to twenty hours.

The so-called *Ficker Diagnosticum* is a suspension of typhoid bacilli which have been killed off by a special process, which has not been made public. The outfit is sold by Merck and is used in the macroscopic application of the test. It consists of a series of small stoppered tubes, a graduated dropping tube, a bottle of the diagnosticum and one of normal salt solution, a small cupping glass and lancet. Cupping glass, rubber stopper, and lancet must first be sterilized by boiling in water. The blood is obtained from the back of the patient by making three or four deep punctures[1] and applying the cupping glass in the usual manner, viz., after placing a few drops of alcohol in the bottom and igniting it and rapidly placing the bottle to the skin before the flame is extinguished. The skin of the back is first cleansed with soap and water, alcohol, and ether. About 1 c.c. of blood is thus drawn, the bottle closed with the rubber stopper and set aside in a cool place until the serum has separated. The test-tube and pipette are sterilized by means of alcohol and ether and the stoppers by boiling in water; 0.1 c.c. of the *clear* serum is now placed in one of the test-tubes, and after washing the pipette with water, alcohol, and ether, diluted with 0.9 c.c. of normal salt solution. A dilution of 1 in 10 thus results. The mixture is well shaken, and 0.1 c.c. placed in a

[1] I find it more convenient to collect the necessary amount of blood from the ear; from 1 to 5 c.c. can be obtained by ordinary puncture without difficulty.

second tube and 0.2 c.c. in a third. With the carefully washed pipette 0.9 c.c. of the diagnosticum is added to test-tube No. 2 and 0.8 c.c. to No. 3. Dilutions of 1 to 100 and 1 to 50 thus result. A further tube (No. 4) receives 1 c.c. of the diagnosticum alone. All tubes are closed, well agitated, and set aside in the dark at room temperature. They are inspected after ten to twelve hours, when, as a rule, a positive reaction can be detected. Sometimes it is necessary to wait for twenty hours; if after that the result is negative it is so reported. If the reaction is positive the bacilli in tubes 2 and 3 will have fallen to the bottom, leaving the supernatant fluid clear, while the control tube 4 remains turbid. All tubes should be viewed against a dark background.

The results which are obtained with Ficker's diagnosticum are very satisfactory. The method has been amply investigated and uniformly indorsed.

The formalized cultures described above can be utilized just as well as the diagnosticum and in the same manner or any other modification that may suggest itself to the individual worker.

Paratyphoid Fever.—As a rule, the serum does not react with the typhoid bacillus, while the organism which appears to be pathogenic in the individual case is agglutinated in a typical manner. Unfortunately, however, the serum of one case will not always react with the organism of a second case; so that the serum reaction will not always make it possible to distinguish the intermediates as a group from typhoid on the one hand, and the bacillus coli on the other. Moreover, it has been shown that the serum of true typhoid may agglutinate the paratyphoid bacillus in higher dilutions even than the typhoid bacillus, although this is probably not usual (Grünberg and Rolly).

Malta Fever.—The diagnosis of Malta fever has been greatly facilitated by the discovery that pronounced agglutination may be obtained with the patient's serum. A positive reaction with a dilution greater than 1 to 30 may be regarded as proof positive of the existence of the disease. As a rule, agglutination can be obtained with a dilution of from 1 to 600 to 700.

The diagnosis of other diseases by the agglutination test is not practicable. For the identification of various bacteria, however, the method is most valuable. This is particularly true in the case of the *cholera bacillus* where the reaction is virtually specific, as group reactions (for related organisms) hardly ever enter into the question. In the case of the *dysentery bacillus* co-agglutination with the typhoid and colon bacillus is not infrequent. For the identification of the *meningococcus* the method is quite valuable; some strains, however, are agglutinated only after twenty-four hours and at a temperature of 56° C. The agglutination test in the case of the tubercle bacillus is beset with technical difficulties which are hard to overcome.

DIAGNOSTIC METHODS DEPENDING UPON COMPLEMENT FIXATION

The Diagnosis of Syphilis.—The Wassermann Reaction.—The Wassermann reaction is based upon the observation that syphilitic blood serum in the presence of certain lipoids will bind complement to such an extent that upon the subsequent addition of hemolytic amboceptor and corresponding corpuscles hemolysis will be prevented to a greater or less extent.

Wassermann was led to the discovery of this reaction by supposing that the blood serum of syphilitic patients contained antibodies of amboceptor nature which would react with appropriate antigen, containing the Spirochæte pallida, in a manner analogous to what was known to take place between bacterial amboceptors and bacterial antigens in the presence of complement. The original concept of the reaction can be schematized as follows:

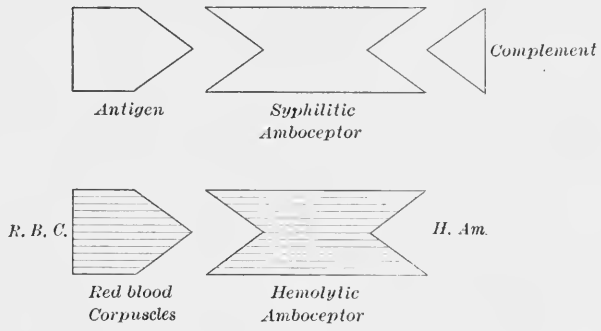


FIG. 62.—Schema illustrating the principle of the Wassermann reaction.

From this diagram it is clear that in the presence of syphilitic amboceptor the complement will become anchored, or fixed, as we usually say, so that it is not available for the hemolytic system R. B. C. and H. Am. when this is subsequently added. Hemolysis is accordingly prevented, if the serum in question comes from a syphilitic patient, while in non-syphilitic cases, in which the syphilitic amboceptor is lacking, the complement is free to act upon the hemolytic system and hemolysis accordingly takes place. Partial hemolysis will occur if the complement is present in excess of the syphilitic antibody.

Basing his work upon the principle just outlined, Wassermann originally used extracts of livers from syphilitic fœtuses as antigen as these are usually rich in the specific spirochetes. Subsequently, it

was ascertained that complement fixation also occurs in the presence of extracts from normal livers and other organs (guinea-pig heart, human heart, ox heart, ox liver, dog kidney, malignant tumors), and that the reacting substance can be extracted with alcohol. It was shown that lecithin, bile salts, and sodium oleate react with syphilitic sera in practically the same manner as do the aqueous and alcoholic organ extracts. These findings render Wassermann's original explanation of the phenomenon as an antigen-antibody reaction, in the sense of Ehrlich untenable, and in spite of numerous investigations the interpretation of its physiologico-chemical significance is as yet an open question. It seems, however, from the evidence at hand that the phenomenon can be fully explained upon the basis of the physical adsorption of complement by the reaction product of a lipoid substance present in the organ extracts and an as yet unknown component of syphilitic blood serum. Whether or not this latter is specific of syphilis has not been definitely ascertained, but seems rather doubtful; it is quite possible that it merely represents a normal component of the blood serum, which in syphilis is present in increased amount.

Regarding the specificity of the Wassermann reaction the evidence seems quite conclusive that outside of syphilis it is rarely met with. In cancer various investigators have obtained a certain degree of complement fixation in a not inconsiderable percentage of cases. It is to be noted, however, that in the majority of the positive cases of this order the degree of fixation is only partial.

Several observers have reported that a certain degree of fixation may be temporarily obtained in from 40 to 50 per cent. of scarlatina cases, while others have not been able to confirm these results.

Outside of these diseases a positive reaction has been described in isolated cases of frambesia, lepra, and sleeping sickness. These exceptional cases, however, do not seriously interfere with the diagnostic value of the reaction in syphilis. Regarding the constancy of the reaction in syphilis, there is now abundant evidence to show that it may be obtained in over 90 per cent. of the cases which are *known* to be syphilitic and in somewhat more than 50 per cent. of the latent cases, the various types giving the following values:

Primary cases	78	per cent.	positive
Secondary cases	90	"	"
Tertian cases (active)	85	"	"
Congenital cases	94	"	"
Cerebrospinal syphilis	81	"	"
Tabes cases	61	"	"
Paresis cases	98	"	"
Latent cases	52	"	"

So far as the effect of antisyphilitic treatment is concerned, it appears that the reaction is less apt to be obtained when this has been

actively carried out for a long period of time; but it has not yet been satisfactorily ascertained whether the reaction remains permanently absent, when once it has been caused to disappear. It is similarly uncertain whether or not it is necessary to continue the treatment until the reaction has disappeared. A great deal of future work is necessary along these lines before any definite rules can be laid down, but there is a growing tendency not to discharge a syphilitic patient from observation until the Wassermann has been negative for two years. (See also section on Syphilis in Part II.)

Method of Wassermann-Bruck (Slightly Modified).—TECHNIQUE. PREPARATION OF THE REAGENT.—1. *Antigens.*—While Wassermann originally advocated the use of saline extracts of syphilitic livers as antigen, it was soon shown that alcoholic extracts gave equally satisfactory results. Subsequent investigations then demonstrated that alcoholic extracts derived not only from *normal liver* tissue, but from heart muscle and kidneys could likewise be used. and to judge from the published evidence, are nearly as serviceable as the extracts derived from syphilitic organs. While this question may still be open to discussion there can be no doubt that the use of syphilitic extracts is not *essential,* and the great majority of laboratory workers the world over have been using alcoholic extracts of normal organs with a greater or less degree of satisfaction up to the present time.

Preparation of Alcoholic Organ Extracts (Michaelis).—If a syphilitic fetal liver be available this should be given the preference. If not, normal human or normal beef heart may be advantageously employed. The material in question is freed from fat and connective tissue as far as possible, passed through a meat hasher, and ground to a mush with clean sand in a mortar. It is then mixed with absolute alcohol in the proportion of 10 c.c. for every gram, and shaken in a closed bottle for a couple of hours, after which it is advisable, though not indispensable, to heat the mixture in a water-bath for an hour at 60° C. The supernatant fluid is finally filtered, and may then be kept at room temperature, best in a dark bottle.

Preparation of Cholesterinized Alcoholic Extracts (Sachs).—Alcoholic extracts of normal heart or liver are prepared as just described, and mixed with a 1 per cent. solution of cholesterin in absolute alcohol, in the proportion of five parts of the alcoholic extract to four parts of the cholesterin solution. While some observers have recommended alcoholic guinea-pig antigen in the past, there can be no doubt that cholesterinized antigen of this order is very apt to become anticomplementary. In this respect our findings are in perfect accord with those of Kolmer and Schamberg.

Preparation of Solutions of the Acetone-insoluble Fraction of Alcoholic Organ Extracts (Noguchi).—Alcoholic extracts of organs (liver

kidney, heart) are prepared as described above, after which the alcohol is driven off by evaporation with the aid of an electric fan, when the residue is taken up with a small amount of ether and treated with five volumes of acetone. The resultant precipitate is allowed to settle to the bottom and after decantation of the supernatant fluid is dissolved in a small quantity of ether, so as to make a saturated solution. This in turn is diluted with nine volumes of absolute methyl alcohol.

Relative Merit of the Different Antigens Just Described.—The Noguchi antigen has the advantage over the plain alcoholic extracts in that its antigenic properties are usually well marked, while its anticomplementary titer is generally distinctly lower. It is, moreover, relatively free from hemolytic properties. For this reason I have personally preferred it very much to the plain alcoholic extracts. Since Sachs has demonstrated, however, that cholesterinized alcoholic extracts derived from *normal* organs are fully as active as plain alcoholic extracts obtained from syphilitic livers, which after all seem to have been the most efficient in the past, there has been a growing tendency to use such "improved" extracts as standard. In my own laboratory we have now examined well over a thousand cases of luetic and non-luetic individuals with cholesterinized beef heart and Noguchi antigen side by side and have found that the former is distinctly more sensitive and more stable than the latter. When positive results are obtained with the cholesterinized antigen and not with that of Noguchi the assumption seems justifiable that irrespective of the history of the patient the existence of a syphilitic infection may be assumed. This conclusion, however, applies only to untreated cases in which a triple plus reaction is obtained. Partial reactions are disregarded, excepting in treated cases, when they are interpreted as indicating incomplete recovery.

Titration of the Antigen.—Since every alcoholic organ extract has anticomplementary properties to a greater or less extent, in other words, as every alcoholic organ extract is capable of fixing a certain amount of complement, it is essential to ascertain to what extent it is necessary to dilute the antigen in order to prevent any noticeable complement fixation in the presence of normal sera, which likewise fix complement to a certain degree. To this end varying dilutions of the antigen are made and combined with the standard amount of complement, when after incubation at 37° to 40° C. for one-half hour, amboceptor and corpuscles are added, and on renewed incubation for the same period of time that dilution is noted with which no fixation and hence complete hemolysis is obtained. *Two-thirds of this strength is then taken as the titer* of the antigen in question. These points will be readily appreciated by a study of the accompanying protocol:

Antigen (dilution).	Saline.	Complement (1 in 10).	Incubation for thirty minutes.	Amboceptor three times the titer strength.	Red cells 5 per cent. emulsion.	Incubation for thirty minutes.	Result.
0.5 c.c. of (3.0 in 10)	0.5	0.5		0.5	0.5		No hemolysis.
0.5 c.c. of (2.0 in 10)	0.5	0.5		0.5	0.5		Partial hemolysis.
0 5 c.c. of (1.5 in 10)	0.5	0.5		0.5	0.5		Complete hemolysis.
0.5 c.c. of (1.0 in 10)	0.5	0.5		0.5	0.5		Complete hemolysis.

The titer of the antigen is thus 1.0 in 10 (viz., two-thirds the stength of 1.5 in 10.

The *dilution of the antigen*, as of all the biological reagents used in the Wassermann reaction, is made with 0.9 per cent. saline. As was first pointed out by Sachs and Rondoin the antigenic value of one and the same extract depends, in the case of some sera, at least, upon the manner in which the dilution is conducted. With plain alcoholic extracts the best results are apparently obtained if the dilution is carried out slowly, so that a maximum opalescence is secured. With the cholesterinized extracts, on the other hand, the method of mixing does not appear to have so much effect, though it is usually found that rapid mixing is the most efficacious (McIntosh and Fildes).

After having determined the titer of the antigen to be used, the preparation is finally tested with a normal serum on the one hand and a well-fixing serum on the other, in order to eliminate the possibility that the fixing titer for syphilitic sera lies too close to the inhibiting value for normal sera. No antigen should of course be used which gives the slightest degree of complement fixation with a normal serum in the titer dose ascertained as described above. The examination in question is conducted by combining one volume of the antigen titer dilution with an equal volume of normal serum, on the one hand, and of a syphilitic fixing serum on the other (after treatment as described sub 5), together with one volume of complement, and then proceeding as described above. The human serum, in other words, is substituted for the one measure of saline (between antigen and complement) as shown in the above protocol.

Durability of the Antigen.—Regarding the durability of the antigenic extracts we have found that cholesterinized beef-heart antigen retains its titer unchanged for at least six months and possibly as long as a year. The Noguchi antigen also is quite stable, while the plain alcoholic extracts deteriorate more rapidly. The least stable in our experience is the cholesterinized guinea-pig-heart extract.

For checking purposes it is our custom to test every serum against at least two cholesterinized beef-heart antigens which differ in their age of preparation by approximately two months. The results should then always tally. If any divergence is noted the titer of the two antigens is determined anew and any one discarded which is found to have deviated from the original figure.

The titer dilution is prepared freshly on every working day.

2. *The Hemolytic Amboceptor.*—To prepare the hemolytic amboceptor a large rabbit is injected on two occasions, seven days apart, with the washed corpuscle corresponding to 30 c.c. of sheep's blood, which must be obtained under aseptic precautions, and after removal of the serum by centrifugation, washed with at least three changes of sterile 0.85 per cent. salt solution. Care should be had each time, after packing down the corpuscles by centrifugation and pipetting off the washings, to stir up the corpuscles in the new portion of saline that is added. Finally, the corpuscles are suspended in an amount of saline, so that the volume injected equals that of the full blood which was originally used. From nine to eleven days later, according to the amboceptor content, which can be readily ascertained by a preliminary test of a few drops of blood, the animal is bled to death, the blood being collected under aseptic precautions. To this end it is convenient to use a test-tube which has been drawn out into a capillary near its closed end, at an angle of about 115 degrees. This is sealed, the open end closed with cotton, and the whole sterilized. After the animal has been anesthetized, the neck is shaved, scrubbed with soap and alcohol, and the carotid dissected out through a median incision. The tip of the capillary is broken off and the tube, moistened with sterile saline, introduced into the vessel, when the blood rises in the collecting tube. The capillary is quickly sealed in a flame and the tube then placed on ice for the serum to separate out; if need be the clot is separated from the walls with a sterile rod. Subsequently, the serum is pipetted off with a sterile pipette, heated for thirty minutes at 56° C., treated with carbolic acid to the extent of 0.5 per cent., and may then be kept in a dark-colored bottle, well corked, on ice. Instead of doing this I find it more convenient to fill small glass beads with about 0.5 c.c. of the serum each, to seal these, and to keep them in an ice box. The addition of carbolic acid is then not necessary.

The titer of the amboceptor should be at least such that 0.5 c.c. of a 1 to 2000 dilution of the amboceptor (in 0.85 per cent. saline) will completely hemolyze 0.5 c.c. of a 5 per cent. emulsion of washed sheep corpuscles (see below), in the presence of 0.5 c.c. of a 1 in 10 dilution of guinea-pig complement (see below), within thirty minutes at 37° C. With the two injections of 30 c.c. of sheep's blood, each, one may obtain a serum which will still hemolyze this quantity of corpuscles in a dilution of 1 to 6000. At other times better results are obtained by giving the rabbit four or five injections of 5, 10, 15, and 20 c. c. of washed corpuscles, in succession, five days apart, the animal being killed when the desired titer has been reached.[1]

[1] During the past year we have desensitized our animals one hour before the second and subsequent injections by the hypodermic administration of a drop or two of the corpuscle emulsion, and have thus obtained somewhat higher titers and rarely lost any animal through anaphylaxis.

Using one of the little beads just mentioned, I make up a 1 to 100 stock dilution which, when kept on ice, will usually retain its titer for many weeks, and is used to make up the higher dilutions on the days when these are wanted. It is best, however, to test it against the complement anew at least once a week, as the activity of the complement varies considerably in different guinea-pigs. In the actual experiment, viz., in the study of the patient's sera, from 2½ to 3 times the completely hemolyzing dose is used.

3. *The Washed Corpuscles.*—The necessary amount of sheep's blood may be procured from a slaughtering house, or if this is not available, a sheep may be kept near the laboratory and can be bled from the ear as occasion demands. For the hemolytic experiment, it is not essential to work aseptically. Whereas, formerly we always secured the blood on the day before it was required, we now work with sterilized blood which is serviceable for from three to four weeks. To this end 300 c.c. of sheep's blood are collected in a mixture of 200 c.c. of a 3.8 per cent. solution of sodium citrate in distilled water, 500 c.c. of a 5.4 per cent. solution of dextrose and 1 c.c. of formalin, the reagent being sterilized for one hour in the steam sterilizer. The mixture is gently agitated while the blood is being collected and placed in the ice-box. A portion is then poured off whenever needed and the corpuscles washed three times with saline, as mentioned above. At last all the fluid is carefully pipetted off; from the remaining corpuscle mush a 2.5 per cent. emulsion is prepared in saline, which corresponds to a 5 per cent. emulsion of the native blood.

4. *The Complement.*—Guinea-pig serum is used as complement. As this is supposedly derived from disintegrating leukocytes, it is recommended to obtain the blood some hours before use. We usually kill the guinea-pig the evening before, by cutting the vessels of the neck, after anesthetizing the animal with ether, under a bell jar. The blood is received in Petri dishes and is kept over night on ice. The following morning the serum is pipetted off; if desired one can then place the clotted blood in centrifuge tubes and obtain still more serum by centrifugation. If it is not practical to kill the animal the evening before, this may be done in the morning of the day on which it is used; it is then placed on ice for two to three hours and the serum then obtained by centrifugalizing the clot. Before use the serum is diluted 1 in 10. The unused portion of the concentrated serum may be kept, frozen, for one or two days, but before further use it must be tested and adjusted to the hemolytic amboceptor as described. Very often it will be found to be inert. In my laboratory, we have set aside special days of the week for complement fixation work, and we then make no attempt to preserve any of the complement.

Where only a few specimens are to be examined at one time it is not necessary to kill the animal. A few c.c. of blood can then be

obtained by puncturing the heart with an antitoxin syringe, under anesthesia. My own preference, however, is to kill the animal.

As I have already indicated, the complement, before use, whether fresh or not, must always be adjusted to the amboceptor. (See Amboceptor.)

5. *The Patient's Serum.*—The required amount of serum can readily be obtained from the ear. This is punctured with a small lancet or tenotomy knife, introducing the blade, at an angle, into the lobule and making a small sweep of the point of the blade without enlarging the skin incision, so as to cut a larger number of capillaries. Enough blood can then be milked out in about five minutes to fill a glass tube 1½ to 2 inches long, and having an inside diameter of ¼ of an inch. The tube is corked and thus brought to the laboratory. The clot is then separated from the walls and the corpuscles packed down by centrifugation. The supernatant serum is pipetted off with Wright pipettes (Fig. 60), placed in tubes similar to those in which the blood is collected and inactivated (complement destruction) by heating for thirty minutes at 56° C.

If larger amounts of blood are desired the use of the Keidel tube will be found convenient. This is pictured in the accompanying illustrations (Figs. 63, 64). The collecting bulb contains a partial vacuum, and fills itself automatically after a vein has been punctured and the sealed end has been nicked off. The neck of the bulbed tube, it will be noted, is wrapped with cotton, on which the protecting tube is slipped, the entire apparatus being sterilized before use by heating for one hour at 165° C.

The veins at the bend of the elbow are brought into prominence by compression of the upper arm with a piece of rubber tubing or a bandage, the skin about the site of the puncture having previously been cleansed with soap and water, alcohol and ether. The protecting tube is withdrawn, the obturator removed from the needle, and the latter plunged into the most prominent vein that presents itself, being introduced in a line parallel to the longitudinal axis of the vessel. The sealed end of the collecting tube is broken off with the fingers (in preference to the use of forceps), when the bulb fills itself in a few moments. While this is going on the tourniquet may be removed, when finally the needle is withdrawn and the puncture sealed with a little cotton and collodion. If the specimen is not to be examined at once the protecting tube is replaced and secured with a little strip of adhesive plaster. The specimen is, of course, sterile, and can be kept for a number of days even at ordinary temperatures, though it is advisable to place it in the icebox as soon as it has been received at the laboratory. The serum is separated from the clot by centrifugation and pipetted off in the usual manner.

A normal serum and an actively fixing specimen from a known case of syphilis should, of course, always be available as controls.

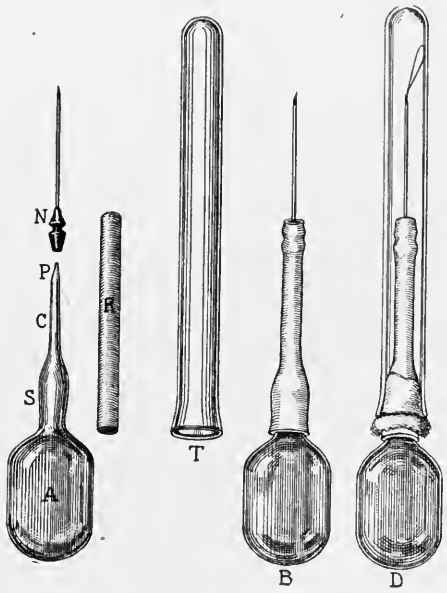


Fig. 63.—*A*, vacuum ampule; *R*, rubber tubing; *N*, needle; *B*, apparatus assembled ready for use; *T*, glass protecting tube; *D*, assembled for sterilizing.

Fig. 64.—Thrusting the needle into the vein, while making traction on the skin to fix the vessel.

While it is a good rule to examine all sera within twenty-four to forty-eight hours after collection, they usually keep for a longer

time without deterioration, and may hence be sent to the laboratory from great distances with perfect safety. All sera which exhibit any trace of spontaneous hemolysis should, of course, be discarded.

Before use, in the actual experiment, all sera are diluted with five volumes of the standard corpuscle emulsion (see above), and incubated in the water-bath for thirty minutes at 37° to 40° C. The tubes are then centrifugated and the clear supernatant fluid pipetted off from the corpuscles. In this manner any natural antisheep amboceptors are removed which otherwise would be added to the hemolytic amboceptor of the hemolysic system that is used, and being an inconstant factor might give rise to erroneous results. A variable amount of complementoid is in this manner removed at the same time (if antisheep amboceptors are present), which would otherwise remain and might likewise interfere with the ultimate result.

The serum, after the treatment just described, is then ready for use and not diluted further.

The Incubator.—If an ordinary incubator is used the duration of each incubation of the specimens is doubled. For this reason most workers now use a water incubator or an ordinary water-bath. The time limits given in the present description of the method have reference to the latter method.

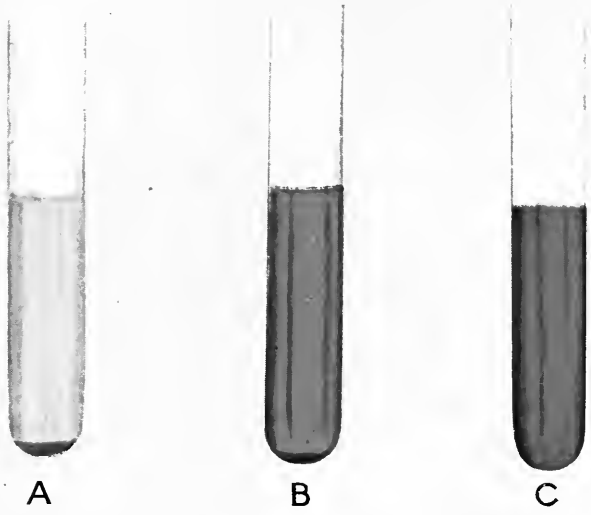
Test-tubes.—The test-tubes which we use measure 4 inches in length by $\frac{3}{8}$ of an inch inside diameter.

Method.—When everything is in readiness the complement and amboceptor are adjusted to one another, using dilutions of 1 to 1000, 1 to 2000, 1 to 3000 to 1 to 6000 of the amboceptor; 0.5 c.c. is our unit of measure, and we accordingly combine 0.5 c.c. of the various amboceptor dilutions with 0.5 c.c. of the complement (1 in 10) and 0.5 c.c. of the corpuscle emulsion (5 per cent.). The tubes are placed in the incubator at 37° C., and frequently shaken. At the expiration of thirty minutes the highest dilution is noted at which complete hemolysis occurs. The amboceptor dilution to be used in the actual experiments is then made $2\frac{1}{2}$ to 3 times as strong. Thus, if complete hemolysis occurred at 1 to 6000, we would use a 1 to 3000 or a 1 to 2000 dilution.

The antigen has been previously tested, as described. With human heart antigen, one can usually use a dilution of 1 in 10.

The titers of the various reagents having thus been ascertained, the experiment proper can now be carried out (E), using 0.5 c.c. of the patient's serum (1 in 6) combined with 0.5 c.c. of complement (1 in 10) and 0.5 c.c. of antigen (1 in 10). At the same time controls (C) are prepared, in which the antigen is left out, so that 0.5 c.c. of each serum is combined with 0.5 c.c. of complement and 0.5 c.c. of saline (in place of the antigen). The E and C tubes properly numbered with the patient's numbers are placed in the

PLATE XIV



Wassermann Reaction.

A, positive; *B*, partial; *C*, negative reaction.

Note undissolved blood corpuscles in *A* partial hemolysis in *B*, and complete hemolysis in *C*.

water incubator for 30 minutes and then receive, each, 0.5 c.c. of the hemolytic amboceptor and 0.5 c.c. of the corpuscles. They are then returned and left until the C tubes are completely hemolyzed. After that Wassermann recommends that they be placed on ice and examined the next morning. I can see no advantage in this delay, and prefer to centrifugalize the tubes and read them at once. If quantitative results are desired one can either take varying dilutions of the patient's serum or of the antigen employed.

Results.—Complete inhibition or absolute fixation is, of course, at once evident from the fact that the supernatant fluid (after centrifugation) is perfectly colorless, the corpuscles being all at the bottom. Partial fixation will show itself by a more or less colored supernatant fluid and a varying number of undissolved red cells at the bottom, while with complete hemolysis there is no sediment of red cells whatever. The results are accordingly noted as $+++$, $++$, $+$, $\pm$, and 0 (Plate XIV).

As regards the *interpretation of the degree of complement fixation* we are inclined to attach *diagnostic* significance only to a triple or double plus reaction, while in a *known* syphilitic undergoing treatment a drop from a double or triple to a single plus may reasonably be interpreted as indicating a diminution in the quantity of the reacting antibody, but as still indicating the existence of an active syphilitic process. A single negative examination has only a limited value, and we are inclined to urge a continuance of the examinations for at least two years, until a patient with a syphilitic past can be discharged from observation. The clinician and the laboratory worker must here work hand in hand.

Regarding the meaning of a single plus reaction in a person who has never had syphilis nothing definite is known. In some cases this may possibly indicate a mild congenital infection, while in others it may be due to antibodies of a different type, which are capable of reacting with the same lipoidal antigen, as the syphilitic antibody.

Anomalous Reactions.—On rare occasions sera are met with which give a complete *fixation even in the absence of antigen.* The significance of such reactions is not altogether clear, but as they have been found only in patients giving a syphilitic history the thought naturally suggests itself that the serum of such individuals may contain the corresponding antigen in sufficient quantity to fix the entire amount of the added complement. It is interesting to note that in a number of instances of this order complete absence of complement has been noted in the patients' sera—an observation which has never been made in a non-syphilitic individual.

Occasionally one meets with individuals who may show marked fixation on one day and no fixation on the next. Sera of this order are spoken of as "paradox sera," and are fortunately rare. The

results are explained by the assumption that such sera contain minimal quantities of antibodies which may be sufficiently large to elicit a reaction at times but then drop below the threshold of reaction.

In this connection it should be borne in mind that free indulgence in alcohol may cause the Wassermann reaction to become temporarily negative.

Modifications of the Wassermann Technique.—NOGUCHI'S METHOD.—Among the numerous modifications of the Wassermann technique that of Noguchi deserves especial consideration. As human serum not infrequently contains antisheep amboceptors in variable quantity, it is clear that its addition to the standard amount of rabbit antisheep amboceptor which is used in the original Wassermann technique might ocasionally obscure a positive reaction; bearing in mind that a relatively small amount of complement is capable of inducing a relatively extensive degree of hemolysis, if hemolytic amboceptor is present in considerable excess. This objection attaching to the original Wassermann technique led Noguchi to substitute the use of a human hemolytic system, *i. e.*, of antihuman amboceptor and of human corpuscles for the antisheep system. In this manner any possible interference on the part of normal antisheep amboceptors would, of course, be obviated. As the same end may be achieved, however, by extracting the patient's serum with sheep corpuscles, as I have recommended above, this objection to the use of the antisheep system may thus be met in a more convenient manner; more convenient, for the reason that sheep's blood can usually be procured more readily for immunization purposes than human blood, that a higher titer can be reached and a more lasting product be obtained.

Another advantage which was claimed for the Noguchi method was the possibility of working with smaller amounts of material and with test papers which had been impregnated with the necessary reagents. As regards the use of the latter it may be said that Noguchi himself has found that complement paper does not retain its activity, and in my own experience the antigen paper likewise cannot be relied upon. All workers, moreover, agree that the complement activity of different guinea-pig sera is by no means constant and that it is imperative to adjust the amboceptor to the complement on every working day. The Noguchi method hence does not shorten the time which is necessary for the test. As far as the use of smaller amounts of material goes, that is merely a question of practice. The majority of laboratory workers have long since abandoned the standard volume of 1 c.c., and in my own laboratory we find no difficulty in working with so small an amount of serum as one single drop, though 0.5 c.c. is our standard volume.

Noguchi's antigen, on the other hand, possesses material advan-

tages over the plain alcoholic extracts which were originally recommended, and I have already advocated its use.

The use of the water-bath or water incubator, which Noguchi recommends, materially lessons the period of incubation as compared with the original directions and can be recommended warmly.

As the Noguchi technique has attracted a good deal of attention, it is described below with the method of preparing the required reagents.

Reagents.—1. *Antihuman Hemolytic Amboceptor.*—This is prepared by injecting rabbits, at intervals of five days, five or six times intraperitoneally with increasing doses (up to 20 c.c.) of washed human corpuscles. The animals are bled to death eight or nine days after the last injection. The titer should be stronger than 0.01 c.c. (*i. e.*, the lowest dilution in which 0.5 c.c. of the serum shall completely hemolyze 0.5 c.c. of washed corpuscles in the presence of 0.025 c.c. of fresh guinea-pig complement, should be 1 to 100). A serum with a titer of 1 to 1000 is recommended, but not imperative.

2. *The complement* is obtained from guinea-pig serum as described, and should be adjusted to the amboceptor before the actual experiment is begun, using 0.04 c.c. complement for two drops of the various dilutions of the amboceptor and 1 c.c. of corpuscles (see below, as also the adjustment of the amboceptor to complement in the original method, p. 166).

3. The *antigen* is prepared as described above (page 163).

4. *Suspension of Human Corpuscles.*—According to Noguchi, this is prepared by mixing one drop of the blood of a normal person with 4 c.c. of normal salt solution. The suspension can also be prepared with the patient's blood, but it can then be used only in combination with the serum of the same patient.

5. *The Patient's Serum.*—This is collected as has been previously described.

Method of Making the Test.—Take six clean test-tubes (10 cm. by 1 cm.). Into the first two of these (A-1 and A-2) place one drop of the serum to be tested, by means of a capillary pipette. Into each of the second two tubes (P-1 and P-2) place one drop of the serum of a syphilitic case, which is known to give a positive reaction (the positive controls). Into each of the third pair of tubes (N-1 and N-2) put one drop of the serum of a normal person (the negative controls). Each one of the six tubes now receives 1 c.c. of the suspension of human red corpuscles and 0.04 c.c. of fresh guinea-pig serum, as complement. Lastly, the -1 tubes of each of the three sets receive the requisite amount of antigen (as determined by a preliminary examination), while the other (-2) tubes go without antigen. After mixing the contents by shaking, all the tubes are placed in the incubator at 37° C. for one hour. Two drops of the antihuman amboceptor are then added to each tube, the contents shaken, and the tubes returned to the

incubator for two hours. Noguchi suggests that the reaction be read from time to time during the next ten to twelve hours, during which the tubes are kept at room temperature. He insists that it is necessary to begin the test with one drop of the patient's serum, and only to use two drops if the reaction is negative, as sera are not infrequent in his experience, in which two drops are inhibitory without the presence of antigen.

As has been pointed out above, complete hemolysis in both tubes of the actual experiment (A-1 and A-2) means a negative reaction. A positive reaction is indicated by hemolysis in the tube without antigen (A-2) and complete or partial inhibition of hemolysis in the one containing both serum and antigen (A-1). A negative reaction will, of course, be shown by the negative controls (N-1 and N-2) and a positive one by the positive controls (P-1 and P-2). In the negative controls complete hemolysis usually occurs within one hour, and sometimes earlier. It is somewhat delayed in the antigen-containing tubes, as compared with those without antigen.

My own experience has led me to break off the experiment when the normal serum-antigen tube shows complete hemolysis on centrifugation. I do not think there is any advantage in waiting longer; indeed, there is a disadvantage, as spontaneous hemolysis may occur in some of the tubes, even in the absence of bacteria.

Complement Fixation in the Diagnosis of Gonorrhea.—Through the investigations of Teagues, Torrey, Schwartz and McNeil, and others it has been established that the principle of complement fixation is practically applicable also in the diagnosis of latent gonococcus infections, a polyvalent extract of gonococci being used as antigen. Schwartz and McNeil prepare this as follows: At least six different strains of gonococci are grown on salt-free veal agar which should be neutral to phenolphthalein. After twenty-four hours the cultures are washed off with distilled water, when the resulting suspension is heated for two hours in the water-bath at 56° C. It is then centrifugalized and passed through a Berkefeld filter. No salt is added to this antigen until it is desired to use it, when it is brought up to 0.9 per cent. strength by adding one part of 9 per cent. saline solution to nine parts of the antigen.

The material is best preserved in glass beads which are heated for half an hour on three consecutive days to insure sterility. Its strength is ascertained by titrating it with a known positive serum, as in the case of the Wassermann reaction. If a patient's serum is not available for this purpose one can conveniently use an antigonococcus serum, prepared by the dealers, which should be diluted to such a point that the antigen gives a reaction with the highest dilution. In greater concentration such sera contain a much larger quantity of antibodies than would ever be found in the serum of a patient.

If a sufficient number of strains of gonococci is not available to prepare a reasonably polyvalent antigen one can make an attempt with one of the corresponding antigens furnished by the dealers. The technique is otherwise in all respects identical with the Wassermann technique (which see).

Complement Fixation in the Diagnosis of Tuberculosis.—The presence of antibodies in the blood capable of reacting with tubercle antigen has been reported by various observers, such as Besredka, Inman, Bronfenbrenner, Anderson, Craig, Zinsser and others. The majority of these observers, however, obtained non-specific reactions also in a not inconsiderable percentage of cases, and notably in syphilis, which malady had already proven an obstacle to Hammer in his attempts to use the complement-fixation method in the diagnosis of tuberculosis, by the aid of an antigen made up from tubercular tissue with the addition of a certain amount of tuberculin. Bronfenbrenner states that he was able to separate a component from Besredka's antigen, which did not react with syphilitic sera and was apparently specific for tuberculosis. But unfortunately none of the observers who have worked with Besredka's antigen (furnished by himself) have been able to prepare an active antigen themselves by following his rather meager working directions. Why this should be is difficult to say. Some workers have suggested that the secret of the method may lie in the necessity of using a polyvalent antigen, and Craig announced, as a matter of fact, that he was able to duplicate Besredka's results by using an antigen made up from several strains of bacilli. His antigen was a shake extract of bacilli grown in alkaline bouillon containing a certain amount of egg, but he expresses the opinion that the method of cultivation is probably an immaterial factor. Still more recently Zinsser has advocated a method of preparing an active antigen, by the aid of which he obtained satisfactory results. In association with Judd I have attempted to repeat the work of previous investigators, but must admit in advance that our results were too inconstant to recommend any of the methods advocated thus far for diagnostic purposes. Evidently much detail work still remains to be done, and I cannot help but feel that the presence of the so-called tubercle wax in the antigens thus far prepared may be the stumbling block that is interfering with active progress. However this may be, I thought it advisable to here introduce one of the methods by the use of which good results have been claimed, so that other investigators may judge for themselves, and perhaps succeed in evolving a more satisfactory plan of work.

Method of Miller and Zinsser.—A number of strains of human tubercle bacilli are grown on Petroff's medium, and young cultures used for the preparation of the antigen. To this end 20 mgrms. of the moist tubercle-bacillus mass are weighed off, in a centrifugalizing

tube, together with 90 mgrms. of table salt. By means of a glass rod, roughened with a file, the mass is triturated for a half-hour. Distilled water is then added to isotonicity, viz., 10 c.c. to the quantities indicated. This is the antigen. The writers advise that just before use the suspension be agitated, the heavier particles allowed to settle, when the material is used as such, without centrifugation or filtration. The writers state that the antigen thus prepared has hardly ever been found anticomplementary in quantities as large as 1 c.c. and has given fixation with positive sera in amounts as low as 0.02 c.c. (the inactivated sera being used in quantities of 0.1 c.c.).

The antigen when kept on ice is said to be quite stable, keeping for at least a month. The titration as well as the reactions were done with one-half the original Wassermann quantities, *i. e.*, 0.5 c.c. doses, using a sensitization of two units of amboceptor and two of complement. The antisheep-hemolytic-rabbit system was used and incubation practised at 37° C. for one hour in the water-bath.

To lessen the danger of the process the tubercle bacilli may be heated for thirty minutes at 60° C., without affecting the delicacy of the reaction.

Results.—Positive reactions were obtained in nearly 100 per cent. of active cases (viz., in 223 of 226 patients). In inactive cases the reaction was almost always negative, in spite of the occasional presence of tubercle bacilli in the sputum of some of the cases. Forty-five positive Wassermann sera were tested; of these only 2 gave positive fixation with the tubercle antigen. One of these two cases could not be followed up; the other was a patient with tubercular peritonitis who had had lues.

Complement Fixation in the Diagnosis of Other Pathological Conditions.—Aside from syphilis and gonorrhea, and tuberculosis, complement fixation has been observed in many other pathological conditions in the presence of suitable antigen; so in typhoid fever, cholera, whooping-cough, cerebrospinal meningitis, pneumonia, in hydatid disease, trichinosis, trypanosomiasis, in cancer, etc. In some of these, like typhoid fever, pneumonia, and cerebrospinal meningitis, where other and less complicated methods of diagnosis are available, the principle of complement fixation will scarcely become applicable as a diagnostic method, while in others it may assume a more important role. It may be mentioned in passing that the hope that cancer might be diagnosed in this manner has not been fulfilled, and for this reason the methods which have been employed to this end in the past have been omitted in the present edition.

DIAGNOSTIC REACTIONS DEPENDING UPON THE PRESENCE OF PROTECTIVE FERMENTS (ABDERHALDEN) IN THE BLOOD

The Diagnosis of Pregnancy.—The Abderhalden Reaction.— Method of Dialysis.—This reaction is based upon the observation of Abderhalden that the blood serum during the pregnant state contains ferments which are capable of bringing about the cleavage of placental proteins to substances which will diffuse through animal membrane and give the biuret reaction.

Necessary Reagents and Apparatus.—1. *The Antigen.*—A *fresh* human placenta is stripped of its membranes, cut into small pieces and kneaded in running water, until the material is macroscopically free from blood. It is then placed in repeated changes of boiling water containing a trace of acetic acid in the first portion (a liter at a time, and boiling for about five minutes), until this no longer gives the biuret reaction with ninhydrin (see below). As this is a *sine qua non* for the success of the experiment, great care should be exercised to attain this end. *Unless the material is absolutely free from substances which give the biuret reaction it is useless to proceed any further.* When once the desired point has been reached the coagulated tissue is most conveniently kept in a glass jar, covered by its last sterile wash water with a thick layer of toluol on top and one of chloroform at the bottom. Generally speaking it may now be viewed as a stable product, but nevertheless it should be tested at frequent intervals, and re-extracted with boiling water, if the biuret reaction returns.

Instead of storing the material as just described, it has been suggested that it may also be dried in a desiccator and then pulverized. For obvious reasons Abderhalden discountenances the use of such material.

In his most recent instructions Abderhalden recommends that immediately before the experiment the requisite quantity of the antigen be removed and boiled for five minutes with five times its volume of water. The material is then filtered through a hardened filter, and 5 c.c. of the filtrate boiled with 1 c.c. of a 1 per cent. aqueous solution of ninhydrin. The material can only be used if no color reaction occurs on standing for thirty minutes. Otherwise it must be extracted with repeated changes of boiling water, until the desired degree of purity has been reached.

2. *The Biuret Reaction.*—While Abderhalden originally advocated either the old copper sulphate test or the new ninhydrin reaction, all workers are now agreed that only the latter should be used, as the first is not sufficiently sensitive for all purposes.

In any event it is not permissible to use the ninhydrin in the actual experiment, if the antigen has been tested with the copper sulphate test only.

The *old biuret test,* as is well known, is conducted by rendering the fluid to be tested strongly alkaline with a strong solution of caustic soda, and then superimposing a weak (0.25 per cent.) solution of copper sulphate, when in the presence of reacting substances a violet purplish ring appears at the zone of contact.

The Ninhydrin Test.—Ninhydrin is triketohydrinden hydrate, a white crystalline substance which is moderately soluble in water. A 1 per cent. solution is employed which is stable. In performing the test 5 c.c. of the fluid under examination are boiled with 0.1 c.c. of the reagent for one minute, when in the presence of reacting substances a purplish-blue color develops, while otherwise the fluid either remains colorless or at most turns a dirty yellow (see plate). In testing the antigen, on the other hand, 1 c.c. of the reagent is used for 5 c.c. of the wash water, as pointed out above.

3. *The Dialysing Tubes.*—These should be impermeable to intact proteins while permitting the diffusion of the corresponding products of cleavage. Abderhalden originally suggested Schleicher and Schüll's Diffusionshülsen No. 579,[1] but as Judd has shown many of these are not sufficiently tight, so that it is necessary in the absence of tubes of constant quality in this respect to test all before use. As the tubes come in lots of twenty-five, it is convenient to test them all at the same time. To this end they are first soaked in water under toluol, until they are pliable. Each tube is then suspended in a small cylinder containing about 10 c.c. of water, covered with a thick layer of toluol, when 0.5 to 1.0 c.c. of fresh blood serum, *free from hemoglobin,* are placed in each tube, the serum in turn being covered with a liberal quantity of toluol, to prevent bacterial decomposition. Thus charged the cylinders are placed in the incubator and kept at body temperature, for from sixteen to twenty-four hours, when the dialysate is tested in portions of 5 c.c each, with ninhydrin as described above (under 2). All tubes which have permitted reacting substances to escape are then cast aside, while the tight ones are carefully cleansed in running water and correspondingly charged with a dilute solution of Witte peptone (1 per cent. solution). After incubation as before, the dialyzate is again tested, when all those tubes which were permeable for reacting substances, are now retained, carefully cleansed in running water and then, as Judd advises, prepared for the experiment proper, as follows: Each dialyzer, filled with distilled water is suspended in 10 c.c. of water in its corresponding cylinder and the latter plugged

[1] Schleicher and Schüll now market a No. 579*a* which is more constant in density, but should be tested nevertheless.

with cotton, when the entire apparatus is sterilized in an autoclave or steam sterilizer in the usual manner, and is then ready for use at any time, it being merely necessary to empty the water contained in the diffusion tubes and to replace it with the necessary reagents. As Judd has shown this is better than to store the dialyzers in a jar of water under toluol, as originally advocated.

4. *The Patient's Serum.*—The necessary amount of blood is procured by venepuncture, using either a Keidel, or a MacRea tube or an aspirating syringe. Two to 3 c.c. of serum should be available, which should be separated from the corpuscles by prolonged centrifugation and used, if possible, on the day on which it was procured. Serum containing hemoglobin in solution or undergoing bacterial decomposition should be discarded. Aseptic working, as far as this is at all possible, is a desideratum, even though not an actual necessity.

The Experiment Proper.—All reagents and apparatus being in rediness, the actual experiment is conducted as follows: One dialyzer (E) is charged with approximately 0.5 gram of the coagulated placental tissue and with 0.5 to 1 c.c. of the patient's serum. A second one (C) receives a similar quantity of the patient's serum only (as control), while a third one (P) is charged with the placental antigen alone, suspended in a corresponding volume of saline. All three dialyzers are guarded with a liberal quantity of toluol, as is also the surrounding water in each cylinder, when all the specimens (best closed with corks) are incubated for twenty-four hours, and are then ready for examination with the ninhydrin, as described, 5 c.c. of the dialyzate being used for each test.

If then a purple color develops in tube E and not in C and P the reaction is positive, while all three remain unchanged or merely turn a dirty yellow, if the reaction is negative. The development of a purple color in P would, of course, indicate that the antigen is not in proper condition, while a similar reaction in C (the proper tightness of the tube being taken for granted) would point to the presence in the serum of reacting substances *per se*, in which case a positive reaction could only be inferred, if tube E showed a much more intense reaction.

Results.—While the results obtained by a number of investigators were at first more or less at variance with those claimed by Abderhalden, it became apparent very soon that these differences were largely due to technical errors, and since the sources of these have become better known the results also have improved, so much so in fact that Abderhalden's claims now seem fully justified, even though the rationale of the reaction as offered by Abderhalden may not be correct. (See section on Pregnancy in the second half of this volume.)

Bronfenbrenner's Modification of the Abderhalden Test.—While the original Abderhalden technique is based upon the view that the reaction is due to the appearance of specific ferments, which bring about the digestion of the corresponding antigen, Bronfenbrenner believes that in the last instance the digestive action of the serum is referable to a proteolytic ferment which is normally present in the serum. He has rendered it quite likely, moreover, that the digestive action which takes place is directed not against the antigen in question but against the normal proteins of the serum. The antigen supposedly unites with the antibody of the specific serum, the presence of which he admits, and as a consequence the antitrypsin which normally inhibits the action of the normal trypsin is absorbed and autodigestion of the serum results. He could show, as a matter of fact, that on bringing serum containing antibodies together with the corresponding antigen at low temperatures the two unite, and that such "sensitized" antigen when subsequently combined with normal non-specific serum will give rise to a specific reaction, whereas the extracted serum when brought together with a fresh portion of antigen is incapable of sensitizing this, so that the latter when subsequently combined with normal serum gives a negative result. Such extracted serum, moreover, when placed in the incubator by itself will promptly undergo self-digestion, while the sensitized antigen when placed in saline undergoes no change. Bronfenbrenner accordingly suggests the following plan of work: 3 c.c. of the specific serum *A* together with 1 gram of the boiled and tested antigen *B* are kept in a test tube on ice for sixteen hours. After centrifugation, the extracted serum *A* is placed in a thimble and dialyzed for sixteen hours at 37° C., when the dialysate is tested in the usual manner and will be found to give a positive result. The sensitized substratum *B* is washed and placed in a thimble with 3 c.c. of saline and incubated for sixteen hours at 37° C. The dialysate will then be found to give a negative reaction. The substratum is now divided into two equal portions of 0.5 gram each B″ and B‴. B″ is treated with 1.5 c.c. of *normal* serum, placed in a thimble and dialyzed for sixteen hours at 37° C., when the dialysate will be found to give a positive reaction. B‴ is likewise treated with 1.5 c.c. of normal serum, but kept for sixteen hours in the ice-box, after which the serum is separated from the substratum, placed in a thimble and incubated, when the dialysate will give a positive reaction, while the substratum after suspension in saline and corresponding incubation will furnish no responding dialyzable products.

3 c.c. of specific serum (A) with 1 gram of boiled substratum (B) in test tube on ice for sixteen hours.

Centrifuged.

Serum A separated and placed in a thimble.

Substratum B washed and placed in a thimble with 3 c.c. of saline.

On ice for sixteen hours.

Ninhydrin test −

Ninhydrin test −
Incubated for sixteen hours at 37° C.

Ninhydrin test +

Ninhydrin test −
Substratum B separated into two parts.

B″

0.5 gram B″ with 1.5 c.c. normal serum incubated in a thimble for sixteen hours at 37° C.
Ninhydrin test +

B‴

0.5 gram B‴ with 1.5 c.c. normal serum placed in a test tube on ice for sixteen hours.
Centrifuged.

Substratum in a thimble with 3 c.c. of saline at 37° C. for sixteen hours.
Ninhydrin test −

Serum in a thimble at 37° C. for sixteen hours
Ninhydrin test +

The necessary controls are shown in the following schema:

0.5 gram substratum (non-sensitized) with 1.5 c.c. normal serum placed in a thimble for sixteen hours at 37°.

Ninhydrin test −

0.5 gram substratum (non-sensitized) with 1.5 c.c. normal serum placed in a test tube on ice for sixteen hours.

Centrifuged.

Substratum in a thimble with 3 c.c. saline at 37° for sixteen hours.
Ninhydrin −

Serum in a thimble at 37° for sixteen hours
Ninhydrin −

Or, in condensed form the entire examination can be conducted as follows:

1.5 c.c. specific serum combined with 0.5 gram boiled substratum in glass tube on ice for sixteen hours.

Centrifuged.

Substratum (sensitized?) plus 1.5 c.c. normal serum in thimble at 37° for sixteen hours.
Ninhydrin test +.

Serum (exhausted) plus 0.5 gram fresh substratum in test tube on ice for sixteen hours.
Centrifuged.
Substratum combined with 1.5 c.c. normal serum in thimble at 37° for sixteen hours.
Ninhydrin test −.

Protective Ferment Reactions in Various Pathological Conditions.—Abderhalden's discovery that protective ferments which are capable of causing the cleavage of placental proteins, can be demonstrated in the blood of pregnant individuals was soon followed by the general observation that corresponding ferments appear under various pathological conditions also, and that these ferments are more or less specific in their nature and appear to be "tuned" to the proteins of definite organs. From the accumulated evidence it would be reasonable to infer, moreover, that the demonstration in a given case of ferments directed against the proteins of a given organ, would warrant the assumption that that organ was involved directly or indirectly in the morbid process underlying the clinical picture.

Especially interesting in this connection is the observation of Fauser (since confirmed by various investigators) that the blood serum of dementia præcox patients contains ferments which are directed against the reproductive glands, such that the serum of female patients reacts with ovaries and that of males with testicles, but not *vice versa*. Incidentally it was found that the serum of the same patients usually also reacted with brain cortex and with thyroid tissue. In general paresis reactions were usually obtained with brain cortex and liver, while at times reactions were also observed with reproductive glands, thyroid, and more rarely with kidney. In none of the so-called functional psychoses, on the other hand (notably in manic-depressive insanity), were ferments demonstrable for any one of the numerous organs which were studied. These observations are, of course, of the greatest possible importance, and doubtless represent an entering wedge along which the exploration of one of the darkest fields of medicine now seems to be possible.

Noteworthy, further, is the observation of Lampé and Papazolu that the blood serum of Basedow patients invariably contains ferments which react with Basedow thyroid and almost always with struma cystica and thymus and in female patients with ovary, while with normal thyroid a reaction was obtained only exceptionally and with other organs at no time, barring one instance in which the serum of the patient reacted with muscle tissue, and in which extensive muscular atrophy existed.

In malignant disease protective ferments have been demonstrated by Frank and Heimann, Gamboroff and others, positive reactions being obtained in a large percentage of cases. Of 50 cases reported by the latter a reaction corresponding to the clinical findings was obtained in all but one. It is noteworthy, moreover, that the serum of carcinoma cases reacted only with carcinoma, and that of sarcoma cases only with sarcoma and not *vice versa*. In this connection it is interesting to note that according to Lindig the serum of

cancer cases may react both with cancer tissue as also with normal placenta. This, however, is denied by Abderhalden, who attributes Lindig's results to faulty technique. Other observers have attained less encouraging results, and I for one do not feel that, in its present form at any rate, the Abderhalden technique can be utilized in the diagnosis of cancer with any reasonable certainty as regards the value of the findings.

Wegener obtained positive reactions in cases of epilepsy in which dementia existed, using brain cortex as antigen.

Manoiloff claims to have obtained specific findings in patients infected with Tænia solium and Ascaris lumbricoides, using the corresponding substracts as antigen, and Voelkel finally reports specific reactions in diphtheria, anthrax, typhoid as well as in infections with trypanosomes and spirochetes,

Technique.—The technique involved in these examinations is essentially the same as that described in connection with the pregnancy reaction, the various antigens which enter into consideration being substituted for the placental tissue and prepared in an analogous manner.

CHAPTER II

THE SECRETIONS OF THE MOUTH

SALIVA

NORMAL saliva is a colorless, inodorous, tasteless, somewhat stringy and frothy liquid, having a specific gravity of from 1.002 to 1.009, corresponding to 4 to 10 grams of solids. The quantity secreted in twenty-four hours amounts to about 1500 grams. An increase is frequently noted in pregnancy, in various neurotic conditions, in tabes, bulbar paralysis, in inflammatory diseases of the mouth, in dental caries, following the administration of pilocarpin, in poisoning with mercury, acids, and alkalies, etc. The quantity is diminished in all febrile diseases, in diabetes, and often in nephritis. The effect of psychic influences upon the secretion of saliva as well as the other glands is well known, an increase or decrease in the flow being produced under various conditions.

In determining whether or not salivation actually exists, the physician should not only be guided by the statements of the patient, but by an actual estimation of the amount secreted within a definite period of time. Nervous individuals not infrequently complain of "salivation," when a direct estimation will show that the amount is not only not increased, but actually diminished.

The reaction is alkaline, the degree of alkalinity corresponding to from 0.006 to 0.048 per cent. of sodium hydrate.

An acid reaction has been noted in various diseases of the intestinal tract, in febrile diseases, and notably in diabetes. An alkaline reaction, however, is the rule even under pathological conditions. Normally an acid saliva is observed only in newly born infants and sucklings.

The reaction of the tongue and the mucous membrane lining the mouth is quite commonly acid early in the morning owing to the production of lactic acid by some of the bacteria which are constantly present in the mouth. The chemical composition is apparent from the appended analyses; the figures correspond to 1000 parts by weight:

Water	995.20	994.20	988.10
Ptyalin[1]	1.34	1.30	1.30
Mucin Epithelium	1.62	2.20	2.60
Fatty matter	..	..	0.50
Sulphocyanides	0.06	0.04	0.09
Alkaline chlorides	0.84		
Disodium phosphate	0.94	2.20	3.40
Magnesium and calcium salts	0.04		
Alkaline carbonates	traces		
Nitrites	traces		

[1] These figures are too high, as they refer to the total precipitate obtained with alcohol.

In order *to demonstrate the presence of sulphocyanides*, it is usually only necessary to heat a few cubic centimeters of the pure saliva, faintly acidified with hydrochloric acid, with a dilute solution of ferric chloride, when a red color will be seen to develop. If necessary, larger quantities are evaporated to a small volume when the test is applied to the concentrated fluid.

To *test for nitrites*, about 10 c.c. of saliva are treated with a few drops of *Ilasvay's reagent* and heated to a temperature of 80° C.; when in the presence of nitrites a red color will develop. The reagent is prepared as follows: 0.5 gram of sulphanilic acid in 150 c.c. of dilute acetic acid is treated with 0.1 gram of naphtylamin dissolved in 20 c.c. of boiling water. After standing for some time the supernatant fluid is poured off and the blue sediment dissolved in 150 c.c. of dilute acetic acid. The solution is kept in a *sealed* bottle.

To *test for ptyalin*, a few cubic centimeters of saliva are collected and added to a solution of starch; the mixture is placed in the warm chamber for five to ten minutes. At first starch gives a blue color when treated with a drop of Lugol's solution or tincture of iodine; as digestion proceeds, a red or violet red is obtained, indicating the presence of erythrodextrin, while later, when achroödextrin only is present, no change in color occurs. The end product, maltose, may be recognized by the fact that it turns the plane of polarization more strongly to the right than glucose; like glucose, it reduces Fehling's solution.

Traces of urea have been found even normally, while increased amounts may be met with in advanced nephritis. Bile pigment and sugar have never been encountered.

Microscopic Examination of the Saliva.—If normal saliva is allowed to stand, two layers will be seen to form, viz., an upper clear and a lower cloudy layer, which latter contains certain morphological elements. Among these, salivary corpuscles, pavement epithelial cells, and microörganisms are found (Fig. 65).

The salivary corpuscles resemble white corpuscles very closely, but differ in their greater size and coarser appearance. The epithelial cells are large, irregular, polygonal cells, provided with well-defined nuclei and nucleoli.

While schizomycetes and moulds are only exceptionally found in the mouth under normal conditions, bacteria are always present in large numbers. Some of these, such as the Leptothrix buccalis innominata, Bacillus buccalis maximus, Leptothrix buccalis maxima, Iodococcus vaginatus, Spirillum sputigenum, and Spirochæte dentium, are always present. Together with other bacteria, these have been found in carious teeth, in abscesses communicating with the mouth and pharynx, and in exudates on the mucous membranes of these parts. In all probability they are non-pathogenic. To this class also belongs the smegma bacillus, which has been encountered

in the saliva, the coating of the tongue, and in the tartar of the teeth of perfectly healthy individuals. The Leuconostoc hominis further is a normal inhabitant of the oral cavity, but occurs in larger numbers in inflammatory diseases (scarlatina, measles, and diphtheria).

In this connection it is interesting to note that, in contradistinction to the bacteria which are only temporarily found in the mouth, the majority of those which are constantly present cannot be cultivated on artificial media.

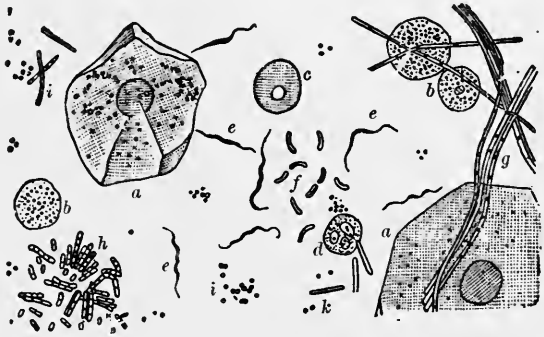
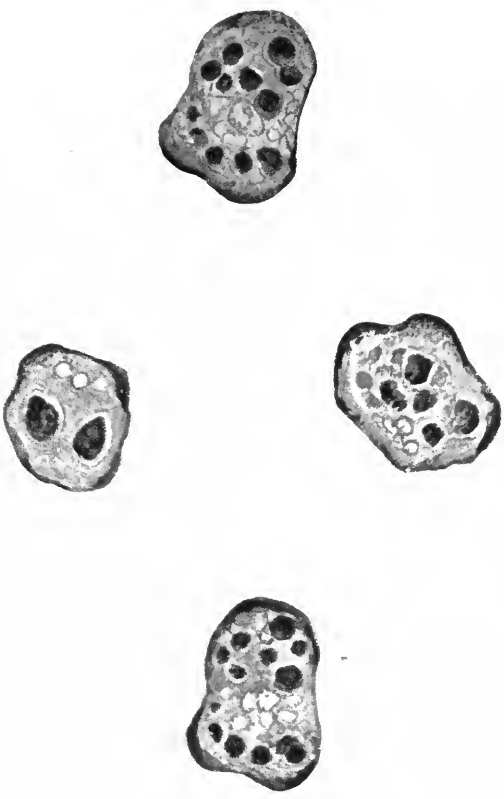


FIG. 65.—Buccal secretion: *a*, epithelial cells; *b*, salivary corpuscles; *c*, fat drops; *d*, leukocytes; *e*, Spirochæte buccalis; *f*, comma bacillus of mouth; *g*, Leptothrix buccalis; *h*, *i*, *k*, various fungi. (Eye-piece III, obj. Reichert, $\frac{1}{15}$ homogeneous immersion: Abbe's mirror, open condensers.) (v. Jaksch.)

Important from a practical standpoint is the fact that a number of pathogenic microörganisms may be found in the throats of *healthy* individuals and may persist for a considerable length of time in persons who have passed through the corresponding infections (infection carriers). This is true especially of the pneumococcus, the streptococcus, the diphtheria bacillus, the influenza bacillus, the catarrhal micrococcus, the meningococcus, and, to judge from recent research, also of the ultramicroscopic organism causing epidemic infantile paralysis. In connection with the pneumococcus it is interesting to note that Types I and II are practically only found either in convalescents from the corresponding types of pneumonia or in persons who have been in contact with such patients, while Type IV is of common occurrence in persons who are enjoying ordinary health and have not been in contact with pneumonia patients. Type III also seems to be quite frequently encountered in such persons.

Regarding the diphtheria bacillus, Welch mentions that virulent organisms were found by Park and Beebe in the healthy throats of 8 out of 330 persons in New York who gave no history of direct contact with cases of diphtheria; 2 of these 8 persons later developed the disease. Non-virulent bacilli were found in 24 individuals of the same series, and pseudodiphtheria bacilli in 27.

PLATE XV



Ameba Pyorrheæ Alveolaris.

Other pathogenic bacteria which may be found in normal mouths are the Micrococcus tetragenus, Streptococcus aureus and albus, the Bacillus pneumoniæ of Friedländer, the Bacillus crassus sputigenus, and the Bacillus coli communis.

ALVEOLAR PYORRHEA

The pus from cases of alveolar pyorrhea quite constantly contains enormous numbers of spirochetes, bacilli of the pseudodiphtheritic type, streptococci, organisms resembling the bacilli of Vincent, amebæ, besides many others which have as yet been very imperfectly described. A number of investigators have attached especial significance to the amebæ, regarding these as the essential cause of alveolar pyorrhea. Two types are usually seen, which differ markedly in size (see Plate XV) and their tendency to engage in phagocytosis. Representatives of the larger form are usually seen containing one or more pus corpuscles, while the others seem to confine their phagocytic activity to bacteria. Their exact position among the amebæ has not yet been definitely established.

TARTAR

In a bit of tartar scraped from the teeth actively moving spirochetes are seen, as well as long, usually segmented bacilli, frequently forming bands which are colored bluish red by a solution of iodopotassic iodide. Leptothrix buccalis, shorter bacilli (which are not colored by this reagent), micrococci, and a large number of leukocytes and epithelial cells which have undergone fatty degeneration, are also found. Infusoria have been met with by Sternberg, P. Cohnheim, v. Leyden, and others.

COATING OF THE TONGUE

A brown coating of the tongue is often observed in severe infectious diseases, and commonly consists of remnants of food and incrustated blood. Microscopically, a large number of epithelial cells, enormous numbers of microörganisms, and a large number of dark, cell-like structures, probably derived from desquamated epithelial cells, are also found. The common white coating of the tongue contains epithelial cells, many microörganisms, and a few salivary corpuscles.

COATING OF THE TONSILS

Of the various organisms which may lead to the production of exudates upon the tonsils and neighboring structures, the most important one, of course, is the diphtheria bacillus; next in their

order of frequency come the Staphylococcus aureus, streptococcus, and possibly the pneumococcus (the distinction being sometimes very difficult), the catarrhal micrococcus, and in fairly rare instances the spirilla and fusiform bacilli of Vincent. In the mucous patches of syphilis, the Spirochæte pallida may be demonstrable, in thrush the Monilia (oïdium) albicans, and in pharyngomycosis leptothrica the Leptothrix buccalis (Fig. 66). In all cases where deposits of any kind are noted in the oral cavity, a portion should be removed by means of cotton swabs, a pair of forceps, or a stout platinum loop, and smears as well as cultures made at once. (For a description of the methods of identification and the special characteristics of the various organisms in question, see the bacteriological appendix.) Besides the various types of bacteria which may thus be encountered, there are pus corpuscles in large numbers, pavement epithelial cells, red blood corpuscles, and cellular detritus.

Fig. 66.—Leptothrix buccalis. (v. Jaksch.)

In the little pyoid masses derived from the crypts of the tonsils, which are occasionally expectorated even by persons in good health, and which are characterized by their stench on being squeezed between the fingers, large numbers of pus cells in all stages of disintegration will be seen, besides broken-down epithelium and bacteria in large numbers, among which the leptothrix buccalis not infrequently predominates. More extensive invasions have been described by Dubler, who noted a leptothrix mycosis involving the pharynx, esophagus, and larynx; and by Baginsky in the case of the pharynx, trachea, and nose.

SPRUE

In cases of sprue a monilia has been described by Bahr and Ashford which the writers regard as the cause of the disease. Regarding the

organism which Ashford discovered in patients in Porto Rico the following description is given: "It reproduces by side buds and terminal conidia from specialized hyphæ, as well as by budding from yeast forms (Fig. 138). The yeast is round, from 2 to 7 μ in diameter, averaging from 3 to 4. Tendency to mycelial formation is seen by the round yeasts enlarging and becoming oval. As the yeasts become larger, many highly refractive bodies of the size of a staphylococcus race incessantly about within the organism, and frequently passing out into the fluid medium in which they grow, may confuse the novice into the belief that a contaminating coccus may be present.

The mycelium is well developed, presenting sparsely bunching hyphæ, sometimes over 1000 micra long. The organism grows well on Sabouraud's glucose agar, 4 per cent., + 2. On this medium bacterial growth is efficiently inhibited even in strokes from feces and plain agar, "the monilia standing out with unfailing precision, is moist, with colonies of sharply contoured lines." The organism is stained best with one of the Romanowsky (methylene azure) mixtures.[1]

[1] For further details see Ashford's paper on Moniliasis (Am. Jour. Med. Sc., November, 1915, p. 680).

CHAPTER III

THE GASTRIC JUICE AND GASTRIC CONTENTS

As the chemical composition of the stomach contents varies with the stage of digestion and the amount and character of the food ingested, it is essential, for purposes of comparison, to make the examination always at a definite time, and best after the administration of a meal of known composition. For this reason certain test meals are employed and the height of digestion chosen as the time at which the stomach contents are procured.

TEST MEALS

The Test Breakfast of Ewald and Boas.—This consists of 35 grams of wheat bread (an ordinary slice) and 400 c.c. of water or weak tea, without sugar. It is best to give this meal to the patient early in the morning, when the stomach is empty—*i. e.*, as a breakfast, and in cases of dilatation or of marked atony, after previous lavage. The gastric contents are obtained one hour later. This is the meal which is most commonly employed in routine work. It may be administered, if so desired, at the physician's laboratory, and can be readily removed through the stomach tube without fear of clogging the eye.

The Test Breakfast of Boas.—This consists of a plateful of oatmeal soup, prepared by boiling down to 500 c.c. one liter of water to which one tablespoonful of rolled oats has been added. A little salt may be used if desired, but nothing more. The contents of the stomach are obtained one hour later. This test meal was devised by Boas in order to guard against the introduction from without of lactic acid, which is present in all kinds of bread. The meal is employed in cases of suspected cancer of the stomach in which a quantitative estimation of lactic acid is to be made, the stomach being washed out completely the night before.

The Test Dinner of Riegel.—This consists of a plate of soup (400 c.c.), a beefsteak (150 to 200 grams), and 150 grams of mashed potatoes. The contents of the stomach are obtained after four hours. The disadvantage of this method lies in the fact that the lumen of the tube is frequently occluded by pieces of undigested meat, a source of annoyance which may be guarded against by using finely chopped meat. Moreover, a positive lactic acid reac-

tion (referable to sarcolactic acid) is obtained in a large number of cases, and entirely irrespective of the amount of hydrochloric acid present. This meal is hence of greater service for gauging the motor power of the stomach than for studying the chemical composition of the gastric juice.

The Double Test Meal of Salzer.—For breakfast the patient receives 30 grams of lean, cold roast, hashed or cut into strips sufficiently small so as not to obstruct the stomach tube; 250 c.c. of milk; 60 grams of rice, and 1 soft-boiled egg. Exactly four hours later the second meal is taken, consisting of 35 to 70 grams of stale wheat bread and 300 to 400 c.c. of water. The gastric contents are withdrawn one hour later. In this manner the gastric juice is not only obtained at the height of digestion, but an idea may at the same time be formed of the motor power of the stomach. Under normal conditions, the organ should contain no remnants of the first meal at the time of examination.

The Stomach Tube.—The stomach tubes in general use are essentially large Nélaton catheters. They should measure from 72 to 75 cm. in length, and be provided with three fenestra, of which one is placed at the end of the tube and two laterally, as near the end as possible. For the purpose of washing out the stomach the tube is connected with a glass funnel.

It is important that the tubes should be thoroughly cleansed in hot water as soon after use as possible. The advice of Boas, moreover, to have special marked tubes for tubercular, syphilitic, and carcinomatous patients should be borne in mind. Patients in whom lavage is to be practised for any length of time should provide their own instruments.

Contraindications to the Use of the Tube.—Of direct contraindications to the use of the tube there should be mentioned the existence of the various forms of valvular disease when in a state of imperfect compensation, angina pectoris, arteriosclerosis of high degree, aneurysm of the large arteries, recent hemorrhages from whatever cause, marked emphysema with bronchitis, acute febrile diseases, etc.

Introduction of the Tube.—The technique of the introduction of the tube should be as simple as possible; the exhibition of complicated bottle arrangements for the purpose of obtaining the gastric juice only adds to the excitement of a nervous patient, and should be avoided. The patient's clothing and floor of the room should be protected from being soiled by material that may be vomited along the sides of the tube, the dribbling of saliva, etc. For this purpose, Türck's rubber bib[1] with pouch may be advantageously employed. Cocainization of the pharynx is not necessary, but may be resorted to in hyperesthetic individuals, a 10 per cent. solution being employed.

[1] Manufactured by G. Tiemann & Co., New York.

The tube, held like a pen, is passed to the posterior wall of the pharynx, the patient bending his head *forward, and not backward,* as is usually advised. The patient is then told to swallow. The tube is pushed inward until a slight resistance is felt when it meets with the floor of the stomach.

At the least sign of cyanosis or of marked pallor the tube should be withdrawn at once, and the patient observed for a day or two before a second attempt is made.

If th gastric juice does not flow at once, the patient is instructed to bear down with his abdominal muscles, and, if this is insufficient, to cough a little. Repeated attempts of this kind will usually bring about the desired result, unless the tube has not been introduced far enough or too far; in the latter case, it will double upon itself, so that its end rises above the level of the liquid. Pressing upon the abdomen with the hands is of no effect (Method of Expression).

Fig. 67.—Boas' bulbed tube.

Aspiration must at times be employed. For this purpose Boas' bulbed tube (Fig. 67) is convenient. The manner in which it is used is the following: The proximal end of the tube, after having been introduced into the stomach, is compressed and the bulb squeezed, when the distal end is clamped and the bulb allowed to expand. A partial vacuum is thus produced and the stomach contents aspirated into the bulb. Usually the tube may then be withdrawn and the bulb emptied into a beaker; or the tube is again compressed proximally, the contents of the bulb expressed and a new portion aspirated, and so on. If enough material is present, direct siphonage can, of course, be established.

In order to *wash out the stomach,* the funnel is filled with lukewarm water or any desired medicated solution, elevated above the head of the patient, and the water allowed to flow. From 500 to 1000 c.c. may be introduced at one time. By depressing and inverting the funnel over a suitable vessel before all water has left the funnel a siphon arrangement is established and the stomach emptied. It is well to measure the returning water as well as the amount introduced. Should the flow diminish or cease before all the water has been removed, the end of the tube probably stands above the level of the liquid, and the flow can be started again by pushing the tube on farther or by withdrawing it a little, as the case may be.

Washing out the stomach soon after the ingestion of a full meal is always a very tedious and annoying if not an impossible procedure, as the fenestra readily become obstructed. Should this occur, the funnel, filled with water, is elevated as high as possible, with a view to overcome the obstruction by hydrostatic pressure; or, if this proves insufficient, the funnel is detached and the obstruction dislodged by means of air, for which purpose a Politzer bag or the bulb of a Boas tube is very convenient.

Amount.—The amount of material which may be obtained from the non-digesting organ normally varies between 1 and 60 c.c. The quantity which can be procured during the process of digestion, on the other hand, varies with the amount of liquid ingested, the time of expression, the size and motor power of the stomach, and the degree of transudation; the process of resorption probably does not play any part, as it has been ascertained that very little water, if any, is absorbed in the stomach.

As a rule, from 20 to 50 c.c. of filtrate can normally be obtained one hour after ingestion of Ewald's test breakfast.

Abnormally large quantities of gastric juice are found practically only in cases of so-called *hypersecretion*, the "Magensaftfluss" of the Germans, which may occur periodically or continuously. Formerly the presence of appreciable quantities of gastric juice in the non-digesting organ was regarded as conclusive evidence of the existence of this condition, but in the light of Schreiber's researches this position can no longer be maintained. The diagnosis should, hence, only be made when in conjunction with the clinical symptoms of hypersecretion from 100 to 1000 c.c. of pure *gastric juice* can be obtained from the non-digesting organ. To this end the stomach should be emptied completely by the tube before retiring, and an examination made on the following morning, no foods or liquids being allowed in the meantime.

In various pathological conditions abnormally large quantities of liquid may be obtained, which cannot be regarded as gastric juice, however. Attention will be drawn to these conditions later on. (See Vomited Material.)

CHEMICAL EXAMINATION

THE TOTAL ACIDITY OF THE STOMACH CONTENTS

The total acidity of the stomach contents at the height of digestion is normally due to three factors, viz., to hydrochloric acid in combination with the albumins of the food, to hydrochloric acid secreted in excess of the amount required to satisfy the albuminous affinities of the meal (which portion we accordingly designate as free hydro-

chloric acid), and to acid salts which are present in small amount in the food ingested. Under pathological conditions certain organic acids (lactic, butyric, acetic) may further enter into consideration and may take the place of the hydrochloric acid, occurring in either the free or the combined state or both.

The time at which hydrochloric acid will appear in the free state depends, *cæteris paribus*, upon the quantity of albumins ingested. With Ewald's test breakfast it appears after thirty-five minutes and reaches its maximum in from fifty to sixty minutes after eating (see curve, Fig. 68). With Riegel's meal the time is longer; it appears after one hundred and twenty to one hundred and fifty minutes in the free state and reaches its maximum after one hundred and eighty to two hundred and ten minutes.

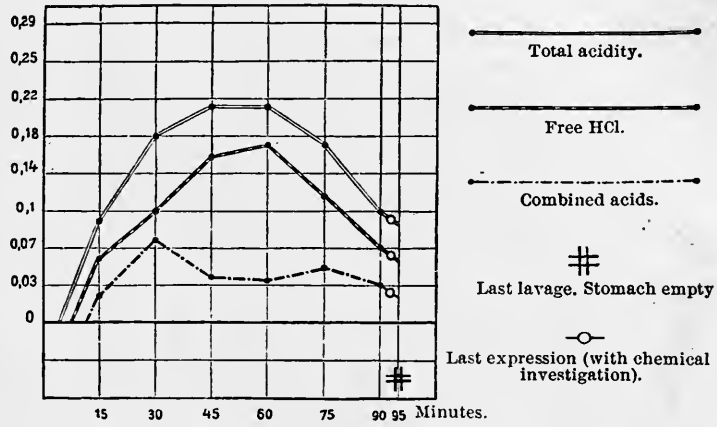


Fig. 68.—Cour e of the acidity of the gastric juice after a test meal of 300 grams of tea and 50 grams of bread. (Schüle.)

Under pathological conditions the amount of free hydrochloric acid, as will be shown, may undergo great variations, diminishing on the one hand to zero, and increasing on the other to 0.5 per cent., or even more.

Method of Determining the Total Acidity of the Gastric Contents.—Five or 10 c.c. of filtered gastric juice are titrated with one-tenth normal solution of sodium hydrate, using 2 or 3 drops of a 1 per cent. alcoholic solution of phenolphthalein as an indicator until a permanent rose color appears. The degree of acidity is then expressed in terms of the number of c.c. of the decinormal solution which would be required to neutralize 100 c.c. of gastric contents.

Example.—Ten c.c. of gastric juice required the addition of 6.5 c.c. of the decinormal solution. For 100 c.c. of gastric juice $6.5 \times 10 = 65$ c.c. would thus be necessary. The total acidity is hence said to be 65.

PLATE XVI

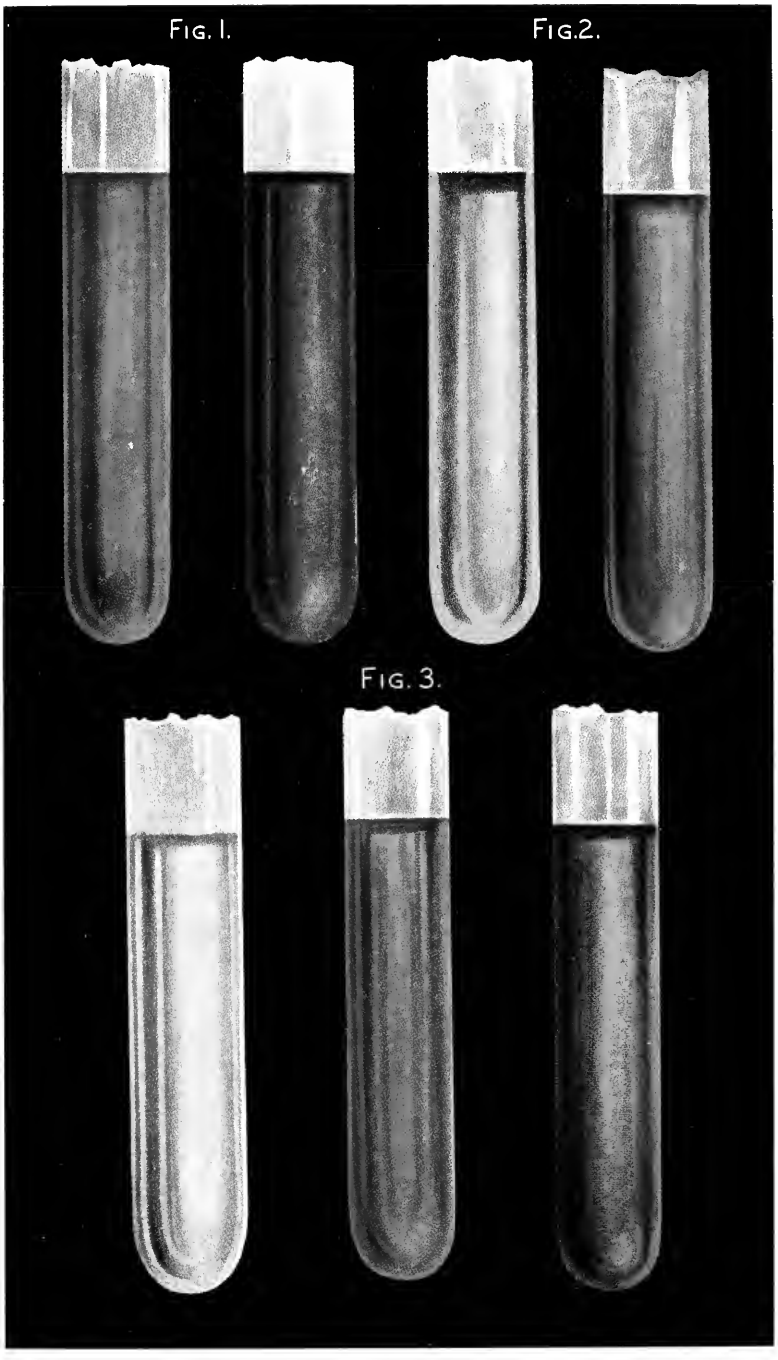


Fig. 1.—Congo Red Test.
Fig. 2.—Dimethyl Reaction.
Fig. 3.—Alizarin Reaction.

Formerly it was customary to express the total acidity in terms of HCl. Since 1 c.c. of the decinormal alkali solution would neutralize 0.00365 gram of HCl, the acidity in these terms, in the chosen example, would be $65 \times 0.00365 = 0.23725$ (*i. e.*, 0.24).

Under normal conditions figures varying from 40 to 60 are usually obtained one hour after the ingestion of Ewald's test breakfast, while in diseases greater variations are observed. In acute and chronic inflammatory conditions of the stomach, as well as in some of the neuroses, the acidity of the gastric contents is, as a general rule, below normal. Higher figures are met with in some cases of ulcer and in some cases of dilatation, but are especially common in neurotic conditions; a degree of acidity corresponding to 90 or even more is then not infrequently observed. Increased acidity, usually associated with hypersecretion of gastric juice, is met with in the so-called *hypersecretio acida et continua* of Reichmann.

Preparation of Decinormal Alkali Solution.—A normal solution of sodium hydrate is one containing the equivalent of its molecular weight in grams—*i. e.*, 40 grams—in 1000 c.c. of distilled water; a decinormal solution will, therefore, contain 4 grams in the same volume of water. This quantity is dissolved in about 900 c.c. and the solution brought to the proper strength by titrating it against a decinormal solution of oxalic acid, which can be made directly by dissolving 6.285 grams of pure oxalic acid in a liter of distilled water. As the decinormal alkali solution has been made purposely too strong, it will be found that less than 10 c.c. will be required to neutralize 10 c.c. of the oxalic acid solution. Supposing we assume that 9.5 c.c. only were required, then every remaining portion of 9.5 c.c. of the decinormal alkali solution would have to be diluted with 0.5 c.c. of water. The solution that has thus been corrected will not materially change its titer for many months, in spite of the gradual appearance of a sediment of sodium silicate.

TESTS FOR INORGANIC ACIDS

Tests for Free Acids.—The Congo Red Test.—This is based upon the fact that solutions of free acids strike a blue color with an aqueous solution of Congo red, which itself is of a peach or brownish-red color (Plate XVII, Fig. 1). For practical purposes it is convenient to keep a small amount of a concentrated solution of the dye on hand and to take a drop or two of this to a small, half a test-tubeful of water when the test is to be made. To this solution, in turn, the filtered gastric contents are added drop by drop. In the place of the Congo solution, filter paper that has been soaked in a moderately strong solution of the dye and then dried and cut into strips may also be employed, but is not quite so sensitive, though satisfactory.

If free acid is shown to be present by the development of a blue color which may vary in intensity from a sky to a deep azure, it is next necessary to determine whether the reaction is due to free hydrochloric acid or, in its absence, to certain organic acids (lactic, butyric, acetic).

Tests for Free Hydrochloric Acid.—The various reagents which may be employed are given below, and are arranged according to their degree of delicacy, viz.:

1. Dimethyl-amino-azo-benzol	0.002	per cent.
2. Phloroglucin-vanillin	0.005	"
3. Resorcin	0.005	"
4. Tropeolin 00	0.030	"

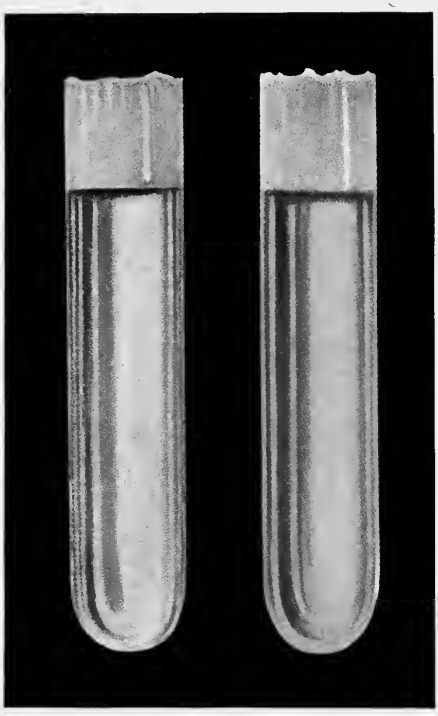
The Dimethyl-amino-azo-benzol Test.—This test has largely replaced the older phloroglucin-vanillin and resorcin tests in the routine work of the clinical laboratory. The delicacy of the reagent is such that the natural yellow color of the indicator is changed to a cherry reddish tinge upon the addition of but 1 drop of one-tenth normal solution of hydrochloric acid in 5 c.c. of distilled water. Its superior delicacy, as compared with the phloroglucin-vanillin and resorcin tests, is apparent from the fact that 5 c.c. of a 0.5 per cent. solution of egg albumin, to which 6 drops of a one-tenth normal solution of hydrochloric acid have been added, still give a positive reaction with dimethyl-amino-azo-benzol, while the phloroglucin-vanillin and resorcin reactions are negative. Organic acids, including lactic acid, yield a red color only when present in amounts exceeding 0.5 per cent. I have further ascertained that *if albumoses are present a cherry-red color is not obtained even though lactic acid be present to the extent of* 1 *per cent.* Loosely combined hydrochloric acid and salts do not produce a red color.

For practical purposes a 0.5 per cent. alcoholic solution is employed; 1 or 2 drops of this are added to a small quantity of the filtered gastric contents; in the presence of free hydrochloric acid a beautiful cherry red develops at once, which varies in intensity with the amount of free acid present (Plate XVII, Fig. 2). In the presence of organic acids a reddish orange color is obtained. The watery solution of the dye itself is of a greenish-yellow color and distinctly fluorescent.

I have used Töpfer's test for many years, and am well satisfied with the results. In teaching students it is well to show the color which one obtains with lactic acid in the presence of albumoses; confusion as to whether or not free hydrochloric acid is present will then not occur.

The Phloroglucin-vanillin Test.—The solution employed contains 2 grams of phloroglucin and 1 gram of vanillin, dissolved in 30 c.c. of absolute alcohol; a yellow color results, which gradually turns a

PLATE XVII



Kelling's Test for Lactic Acid.

dark golden red, changing to brown on prolonged exposure to light. The solution should, therefore, be kept in a dark-colored bottle. Lenhartz suggests the use of separate solutions of phloroglucin and vanillin, 1 or 2 drops of each being employed in the test. Boas recommends a solution of the phloroglucin and vanillin, in the proportions indicated in 100 grams of 80 per cent. alcohol, and claims that the reagent is then still more sensitive and more stable. If a few drops of gastric juice, or even of the unfiltered gastric contents, containing 0.05 per cent. or more of free hydrochloric acid are treated with the same number of drops of the reagent, no change in color results, but upon slow evaporation—*boiling and rapid evaporation are to be avoided*—a general rose tint or fine rose-colored lines develop, which are characteristic of the presence of the free acid.

For practical purposes it is best to carry on this slow evaporation on a thin porcelain butter dish, the porcelain cover of a crucible, or in a small evaporating dish of the same material. The color obtained in the presence of free hydrochloric acid is a rose color in every instance, and varies in intensity with the amount of acid present. A brown, brownish-yellow, or brownish-red color always indicates that excessive heat has been applied or that free hydrochloric acid is absent.

Organic acids do not produce the reaction, nor is it interfered with by their presence, or that of albumins, peptones, or acid salts.

A phloroglucin-vanillin test paper, prepared by soaking strips of filter paper, free from ash, in the solution and drying them, may also be employed. If a strip of this is moistened with a drop of gastric juice and gently heated in a porcelain dish, a rose color will develop in the presence of free hydrochloric acid, and does not disappear upon the addition of ether.

The Resorcin Test.—The solution consists of 5 grams of resublimed resorcin and 3 grams of cane sugar dissolved in 100 grams of 94 per cent. alcohol. It is equally as delicate as the phloroglucin-vanillin solution and has the advantage of greater stability; 5 or 6 drops of gastric juice are treated with 3 to 5 drops of the reagent and slowly evaporated to dryness over a small flame, when a beautiful rose- or vermilion-red mirror will be obtained, which gradually fades on cooling. If the reagent is employed in the form of a test paper, a violet color at first develops, which upon the application of heat turns brick red and does not disappear on treatment with ether.

The presence of acid salts, organic acids, albumins, or albumoses does not interfere with the reaction.

The Tropeolin Test.—Tropeolin 00, when employed according to the method suggested by Boas, is a very reliable reagent, indicating the presence of 0.02 to 0.03 per cent. of free hydrochloric acid; 3 or 4 drops of a saturated alcoholic solution of tropeolin 00, which has a brownish-yellow color, are placed in a small porcelain dish or

cover, and allowed to spread over the surface. A like amount of gastric juice is added and likewise allowed to flow over the surface of the dish; upon the application of gentle heat a beautiful lilac appears, which is said to be characteristic of free hydrochloric acid.

A tropeolin test paper may also be prepared by soaking filter paper, free from ash, in the alcoholic solution, and then drying and cutting it into strips. A few drops of gastric juice containing free hydrochloric acid produce a more or less pronounced brown color upon this paper, which turns lilac or blue upon the application of gentle heat. Organic acids, when present in large amounts, likewise produce a brown color, but this disappears on heating, and a lilac or blue color does not result.

For ordinary purposes this test is sufficient, and recourse need only be had to the more delicate reagents when a negative or a doubtful result is obtained.

The Combined Hydrochloric Acid.—It has been pointed out elsewhere that hydrochloric acid will only appear in the free state after all basic affinities have been saturated. For this reason combined hydrochloric acid must of necessity be present after the administration of a test meal if free acid can be demonstrated. If the contents are withdrawn too early, free acid will be absent, while hydrochloric acid in combined form may be present in normal amount, considering the stage of digestion. From the mere absence of free hydrochloric acid it is hence not justifiable to infer that no hydrochloric acid has been secreted. Under pathological conditions it may happen that while the stomach has lost the power to furnish a sufficient amount of hydrochloric acid to satisfy the albuminous affinities of a large meal and to subsequently appear in the free state, enough can be furnished to meet the demands of a small meal. In any case then where free hydrochloric acid is not found, it is important to ascertain whether no hydrochloric acid at all has been secreted. To this end the method of Martius and Lüttke may be employed (see below). For routine work, however, this method is too complicated, and for such purposes Töpfer's method will be found most convenient.

Quantitative Estimation of the Hydrochloric Acid of the Gastric Juice.—Töpfer's Method.—The free and combined hydrochloric acid is most conveniently estimated according to Töpfer's method, which is both simple and sufficiently accurate for clinical purposes.

In this method the total acidity (*a*) of a given amount of gastric juice—*i. e.*, the acidity referable to the presence of free hydrochloric acid, combined hydrochloric acid, acid salts, and any organic acids that may be present—is first determined (lactic acid and the fatty acids, if present, need not be removed), using phenolphthalein as an indicator. This is followed by a determination of the acidity referable to free acids and acid salts in another sample of gastric juice

(*b*), using alizarin (alizarin monosulphonate of sodium) as an indicator. The difference between *a* and *b* will indicate the amount of the combined acid. The free hydrochloric acid (*c*) finally is estimated with dimethyl-amino-azo-benzol as an indicator, the difference between *a* and $b+c$ giving the acidity referable to organic acids and acid salts.

The solutions required are the following:

1. A decinormal solution of sodium hydrate.
2. A 1 per cent. alcoholic solution of phenolphthalein.
3. A saturated aqueous solution of alizarin
4. A 0.5 per cent. alcoholic solution of dimethyl-amino-azo-benzol.

Three separate portions of 5 or 10 c.c. of filtered gastric juice are measured into three small beakers or porcelain dishes. To the first portion 1 or 2 drops of phenolphthalein are added, when it is titrated with the one-tenth normal solution of sodium hydrate until a permanent pink color is obtained.

To the second portion 3 or 4 drops of the alizarin solution are added, when it also is titrated with the one-tenth normal solution of sodium hydrate, until a pure violet color is obtained (Plate XVII, Fig. 3).

In the third portion the free hydrochloric acid is titrated, after the addition of 3 or 4 drops of the dimethyl-amino-azo-benzol, until the last trace of red—in the presence of free hydrochloric acid—has disappeared, and the color has become distinctly greenish yellow (Plate XVII, Fig. 2). The results are then calculated as in the following example:

Ten c.c. of gastric juice, using phenolphthalein as an indicator, required 6 c.c. of the one-tenth normal solution in order to bring about the end reaction, while a like amount titrated in the same manner with alizarin required 3 c.c. The difference between 6 and 3 indicates the number of cubic centimeters necessary to neutralize the amount of hydrochloric acid in combination with albuminous material. In the estimation of the free hydrochloric acid 2.3 c.c. of the one-tenth normal solution were required.

The results can then be tabulated as follows:

Total acidity (per 100 c.c. stomach contents)	60
Alizarin acidity	30
Combined hydrochloric acid	30
Free hydrochloric acid	23
Total physiologically active hydrochloric acid	53
Salts	7
Total	60

If not enough gastric juice is available for three separate titrations, one can estimate the free hydrochloric acid in one portion of 5 c.c.

with dimethyl as an indicator, and proceed at once to the total acidity in the same example. To this end phenolphthalein is added after the primary titration and the titration continued for the total acidity as usual. The first value will give the free hydrochloric acid, and this plus the second value the total acidity.

Deficit of Hydrochloric Acid.—When hydrochloric acid is absent it is customary to indicate the deficit in terms of $\frac{n}{10}$ hydrochloric acid in a manner perfectly analogous to the method just now described, viz., 10 c.c. of gastric juice are treated with a few drops of dimethyl and then titrated with $\frac{n}{10}$ hydrochloric acid until the red hydrochloric acid reaction appears. If 1 c.c. was necessary to this end the hydrochloric acid deficit would be 10.

The Method of Martius and Lüttke (Modified).—This method is equally exact, but requires a greater expenditure of time. It is based upon the fact that upon incineration of the gastric juice the free hydrochloric acid and that loosely combined with albuminous material escape, while the chlorine in combination with inorganic bases remains in the mineral ash unless a very intense heat is applied for some time. By subtracting the amount of chlorine present in the latter form from the total amount, the quantity in combination with albuminous material and that occurring as free acid will be found. The total acidity of the gastric juice is then determined, and that referable to the presence of the free and combined hydrochloric acid subtracted, the difference giving the amount of organic acids and acid salts. By determining the acidity due to the presence of free hydrochloric acid according to Töpfer's method, and deducting the amount found from that referable to the presence of free and combined hydrochloric acid, the amount of the latter is obtained.

Reagents required:

1. A solution of silver nitrate in nitric acid of such strength that 1 c.c. shall represent 0.00365 gram of hydrochloric acid.
2. Liquor ferri sulphurati oxydati.
3. A decinormal solution of ammonium sulphocyanide.
4. A one-tenth normal solution of sodium hydrate.
5. A 1 per cent. alcoholic solution of phenolphthalein.
6. A 0.5 per cent. alcoholic solution of dimethyl-amino-azo-benzol.

Preparation of the solutions:

1. The silver nitrate solution. As a solution is required of such strength that 1 c.c. shall be equivalent to 0.00365 gram of hydrochloric acid, the amount of silver nitrate that must be dissolved in 1000 c.c. of water is ascertained in the following manner: Since 169.66 (molecular weight) parts by weight of silver nitrate combine with 36.5 parts of hydrochloric acid (molecular weight), the amount of silver nitrate required for each cubic centimeter is found from the equation:

$$169.66 : 36.5 : : x : 0.00365;\ 36.5\ x = 0.6192590;\ x = 0.0169.$$

In 1 c.c. of the silver solution 0.0169 gram of silver nitrate must thus be present, or 16.9 grams in the liter. This quantity, or roughly 17 grams, is weighed off and dissolved in 900 c.c. of a 25 per cent. solution of nitric acid. To this solution 50 c.c. of the liquor ferri sulphurati oxydati are added. The solution is then brought to the proper strength by titration of a known number of cubic centimeters of a one-tenth normal solution of hydrochloric acid and correcting as usual (see below).

2. The ammonium sulphocyanide solution. A normal solution of ammonium sulphocyanide contains 75.98 grams (molecular weight) per liter, and a decinormal solution 7.598 grams. This quantity, or roughly 8 grams, is dissolved in about 900 c.c. of water and the solution brought to the proper strength by titrating a known number of cubic centimeters of the silver nitrate solution, when each cubic centimeter should correspond to 1 c.c. of the silver solution—*i. e.*, to 0.00365 gram of hydrochloric acid. It is corrected as described elsewhere (see below).

METHOD.—1. To determine the total amount of chlorine present: 10 c.c. of gastric juice—Martius and Lüttke make use of the unfiltered gastric contents—are measured into a small flask bearing a 100 c.c. mark, and treated with an excess of the one-tenth normal solution of silver nitrate. Experience has shown that 20 c.c. are sufficient. The mixture is agitated and allowed to stand for ten minutes. Distilled water is then added to the 100 c.c. mark; the mixture is agitated once more and filtered through a dry filter into a dry beaker; 50 c.c. of the filtrate are titrated with the one-tenth normal solution of ammonium sulphocyanide until the blood-red color which appears upon the addition of every drop—due to the formation of ferric sulphocyanide—no longer disappears on stirring. By multiplying the number of cubic centimeters of the ammonium sulphocyanide solution used by 2 (the number of cubic centimeters that would have been necessary for the precipitation of the excess of silver in 100 c.c.) and deducting the result from the number of cubic centimeters of the one-tenth normal solution of silver nitrate employed, viz., 20, the number of cubic centimeters of the latter solution is found which was necessary to precipitate the chlorine in 10 c.c. of the gastric juice. As 1 c.c. of the solution represents 0.00365 gram of hydrochloric acid, it is only necessary to multiply this figure by the number of cubic centimeters used in precipitation of the chlorine. The resulting value, *T*, expresses the total amount of chlorine present.

As a general rule, it is not necessary to decolorize the gastric juice. If desired, however, 5 to 15 drops of a 5 per cent. solution of potassium permanganate may be added to the 10 c.c. employed after the mixture has stood for ten minutes.

2. Determination of the amount of chlorine in combination with inorganic bases, *F:* 10 c.c. of the filtered gastric juice are carefully evaporated to dryness in a platinum crucible, on a water bath or upon a plate of asbestos, in order to avoid sputtering (as the heat applied in the process of incineration is not very intense, a porcelain crucible may also be employed). The residue is then carefully incinerated over an open flame, the process being carried only to the point where the organic ash no longer burns with a luminous flame. Intense heat should be avoided, as the chlorides are volatilized upon the application of red heat. On cooling, the ash is moistened with a few drops of distilled water and mixed with a stirring rod, when the residue is extracted in separate portions with 100 c.c. of hot distilled water and filtered. This amount is usually sufficient to dissolve all the chlorides present. If any doubt should exist, however, it is only necessary to add a drop of the silver solution to a few drops of the last portion of the filtrate: the formation of a cloud, referable to silver chloride, will necessitate still further washing. The whole filtrate is then treated with 10 c.c. of the one-tenth normal solution of silver nitrate, and the amount consumed in the precipitation of the chlorides determined by titration with the one-tenth normal solution of ammonium sulphocyanide, as described above. The hydrochloric acid present in combination with inorganic bases is thus determined. The difference between the amount of hydrochloric acid in combination with inorganic bases and the total amount of chlorine in terms of hydrochloric acid will then indicate the amounts of the free and of the combined hydrochloric acid, which are termed L and C respectively; hence $T-F=L+C$.

3. The total acidity in terms of hydrochloric acid is further determined according to the method given elsewhere (see p. 164) and indicated by the letter A. The difference between the total acidity and the amount of free and combined hydrochloric acid will represent the amount of organic acids and acid salts, O; hence $O=A-(L+C)$.

The free hydrochloric acid finally is determined according to the method of Töpfer. The difference between the value thus found and that expressing the amount of free and combined hydrochloric acid will indicate the amount of the latter; hence $(L+C)-L=C$.

Variations in the Hydrochloric Acid Contents of the Gastric Juice.—Clinically, it is necessary to distinguish between euchlorhydria, or the secretion of a normal amount of free hydrochloric acid (0.1 to 0.2 per cent.), hypochlorhydria, or the secretion of a deficient amount (less than 0.1 per cent.), hyperchlorhydria, in which more than 0.2 per cent. is found, and anachlorhydria, in which no hydrochloric acid at all is secreted.

Euchlorhydria.—Euchlorhydria, when associated with clinical symptoms pointing to gastric derangement, is most commonly observed in neurasthenic individuals. Chronic gastritis can always be excluded in the presence of a normal amount of free acid. It may be associated with a certain degree of atony. It was formerly thought that a normal amount of acid would preclude the diagnosis of ulcer, but it is known that this association is quite possible. The same is seen in pyloric stenosis due to a healed ulcer.

Hypochlorhydria.—Hypochlorhydria is associated with all those diseases in which the secretory elements have been more or less damaged, as the result of general disease (anemia, chronic heart and renal lesions, phthisis, chronic icterus, many febrile diseases), or of local disease, as in subacute and chronic gastritis, in some cases of ulcer of the stomach or the duodenum, in incipient carcinoma, and in certain cases of dilatation and atony. The withdrawal of chlorides from the food will also lead to a diminished production of hydrochloric acid.

Anachlorhydria.—Not many years ago it was thought that the absence of free hydrochloric acid was pathognomonic of carcinoma of the stomach. This view was soon abandoned, however, as it was shown that cases of carcinoma occur in which hydrochloric acid is not only present, but present in excessive amounts. This is true especially of those cases in which the malignant growth has started upon the base of an old ulcer. It is noteworthy, moreover, that in early cases of carcinoma, even in the absence of ulcer, hydrochloric acid may at times be demonstrable and then disappear for days and weeks. It was furthermore shown that anachlorhydria exists in almost all cases of advanced chronic gastritis, in pernicious anemia (gastric anadeny), and is a fairly common occurrence in neurasthenic and hysterical individuals. In these cases, periods of ana-, hyper-, and hypochlorhydria may alternate apparently without cause. In the acute febrile infections also anachlorhydria is not uncommon.

Hyperchlorhydria.—Hyperchlorhydria (acid stomach, gastroxynsis) is very common in neurotic individuals, where it may alternate with hypo- and anachlorhydria. The same is seen even normally during menstruation. Associated with a continuous hypersecretion of gastric juice, it constitutes the neurosis known as *hypersecretio acida et continua* (gastrosuccorrhœa acida). Hyperchlorhydria is also of frequent occurrence in cases of gastric ulcer, and may even occur in carcinoma, notably in those cases in which, as stated above, the newgrowth has started from an older ulcer. Regarding the frequency of hyperchlorhydria in ulcus, there can be no doubt that this is found in the majority of cases. Normal values, however, are by no means uncommon, and in some instances the amount of hydrochloric acid may be diminished.

Hyperchlorhydria is also met with in passive congestion of the

stomach (Schreiber's so-called "stagnant stomach"), in certain types of mental disease, in the early stages of chronic gastritis, during migraine attacks, etc.

THE ORGANIC ACIDS OF THE STOMACH CONTENTS

Lactic Acid.—Mode of Formation and Clinical Significance.—The normal occurrence of lactic acid in the stomach during digestion was formerly regarded as an established fact and generally ascribed to the action of lactic acid producing organisms which had been swallowed and which could exercise their activity so long as hydrochloric acid did not appear in the free state.

Martius and Lüttke, however, employing the method already described, found "that the accurately determined curve of acidity referable to hydrochloric acid coincided in all respects, even at the beginning of the process of digestion, with the curve referable to the total acidity," so that lactic acid as a physiological constituent could not have been present. The researches of Boas, moreover, prove beyond a doubt that in physiological conditions no appreciable amounts of lactic acid are formed during the process of digestion, and that the traces of lactic acid found after an ordinary meal have been introduced into the stomach as such. It is known that lactic acid is present in various kinds of bread, and it is, hence, not permissible to make use of any test meal containing lactic acid when the question as to its formation in the stomach is to be considered. For these reasons Boas suggests the use of simple oatmeal soup to which salt only has been added. (See Boas' Test Meal.) For practical purposes this is probably not always necessary, as the small amount of lactic acid found after Ewald's test breakfast may usually be disregarded; an increased amount can be referred directly to pathological conditions.

Under pathological conditions notable amounts (0.1 to 0.4 per cent.) of lactic acid are met with when stagnation of the gastric contents occurs as a result of motor insufficiency, in the absence of or with a diminished secretion of hydrochloric acid. It is hence a common symptom of carcinoma of the stomach. It was, indeed, at one time thought that carcinoma was the only disease in which a notable lactic acid production took place, but experience has shown that the same may occur in benign cases of pyloric stenosis and gastric insufficiency. Such findings, however, are uncommon, and high lactic acid values may still be regarded as strongly suggestive of malignant disease, especially when repeatedly observed. Early in the disease it appears that periods of chlorhydria and lactic acid production may alternate, and it is desirable that this phase of the problem more particularly should receive attention.

In cases in which carcinoma has developed upon the basis of an

PLATE XVIII

Amebæ Fed with Neutral Red and Containing Phagocytes and Red Cells.

old ulcer, lactic acid may be absent and hydrochloric acid present in increased amount.

In every case in which lactic acid is found the stomach should be thoroughly washed out in the evening and no food allowed until the following morning. Boas' test meal is then given and the examination repeated. If the presence of lactic acid can thus be established on repeated examination, even if a normal condition or hyperchlorhydria can be demonstrated in the interval, an exploratory incision is justifiable.

It should, finally, be mentioned that only that form of lactic acid which results from fermentative processes is of interest in this connection, and not the sarcolactic acid contained in meat. For this reason the demonstration of lactic acid after a meal of meat is of no diagnostic significance, so far as the question of carcinoma goes.

Tests for Lactic Acid.—KELLING'S METHOD (AUTHOR'S MODIFICATION).—This test is best performed in the following manner: To a test-tubeful of water a drop or two of a moderately strong solution of the sesquichloride of iron is added, so that the liquid is barely colored. One-half is then poured into a second tube and serves as control. A small amount of the gastric filtrate is added to the other specimen, when in the presence of lactic acid a distinct yellow develops at once, which appears the more marked when compared with the nearly colorless control. This test is very delicate and to be preferred to the older method of Uffelmann (Plate XVIII).

UFFELMANN'S TEST.—Heretofore Uffelmann's reagent was almost exclusively employed in testing for lactic acid, but everyone who has had occasion to make frequent use of this reagent in clinical work must have been struck with the uncertainty of the results so often obtained. In a large majority of the cases, particularly if Ewald's test breakfast is employed, a characteristic reaction—*i. e.*, the occurrence of a lemon or canary-yellow color—is not seen, notwithstanding the presence of lactic acid, but a pale yellow, brownish, grayish-white, or even gray color is obtained instead, often leaving in doubt whether lactic acid is present or not. Aside from doubtful results, the value of the test is greatly diminished by the fact that glucose, acid phosphates, butyric acid, and alcohol give the same reaction, and that in the presence of such amounts of hydrochloric acid as are found at the height of normal digestion lactic acid is not indicated by the reagent. All these difficulties have long been appreciated, and in order to obviate at least some of them it was proposed to apply the test to an aqueous solution of the ethereal extract of the gastric contents:

To this end 5 or 10 c.c. of the filtered gastric juice are extracted by shaking with from 50 to 100 c.c. of neutral sulphuric ether in a stoppered separating funnel for about twenty or thirty minutes; the ethereal extract is then evaporated on a water bath or the ether

distilled off (*no flame*). The residue is taken up with from 5 to 10 c.c. of distilled water and tested as follows: 3 drops of a saturated aqueous solution of ferric chloride are mixed with 3 drops of a concentrated solution of pure carbolic acid and diluted with water until an amethyst-blue color is obtained; to this solution a portion of the ethereal extract is added, when in the presence of 0.1 per cent. or more of lactic acid a lemon or canary-yellow color is obtained.

Strauss' Method.—Instead of evaporating the ether as in the above method, the ethereal extract may be directly examined by shaking with a solution of ferric chloride, as suggested by Fleischer. Making use of this principle, Strauss has constructed an apparatus (Fig. 69) which will be found very convenient, and which permits of roughly determining the amount of lactic acid present. The instrument is essentially a separating funnel of 30 c.c. capacity, bearing two marks, of which the one corresponds to 5 c.c., the other to 25 c.c. The apparatus is filled with gastric juice to the mark 5, when ether (free from alcohol) is added to the 25 c.c. line. After shaking thoroughly, the *separated* liquids are allowed to escape by opening the stopcock until the 5 c.c. mark is reached. Distilled water is then added to the 25 mark, and the mixture treated with 2 drops of the officinal tincture of ferric chloride, diluted in the proportion of 1 to 10. Upon shaking, the water will assume an intensely green color if more than 1 pro mille of lactic acid is present, while a pale green is obtained in the presence of from 0.5 to 1 pro mille. The tincture of iron should be kept in a dark-colored dropping bottle of about 50 c.c. capacity.

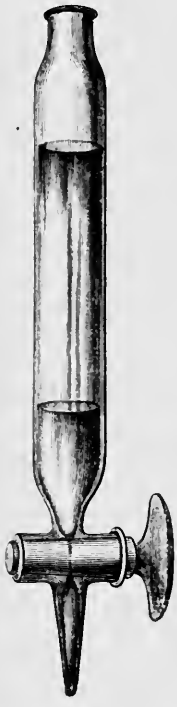


Fig 69.—Strauss' apparatus for the approximative estimation of lactic acid.

It will be observed that only large amounts of lactic acid, which alone are of importance from a diagnostic point of view, are indicated by the apparatus. Small amounts, as those introduced with Ewald's test breakfast, or referable to lactic acid fermentation in the mouth, are not indicated, so that confusion as to the presence or absence of the acid can never arise.

Quantitative Estimation of Lactic Acid According to Boas' Method.—The patient's stomach should be thoroughly washed out before the test meal (Boas') is introduced.

PRINCIPLE.—The principle of the method is based upon the fact that on treating a solution of lactic acid with a strong oxidizing agent and heating, the lactic acid is decomposed into formic acid and and acetic aldehyde according to the equation:

$$CH_3.CH(OH).COOH = CH_3.CHO + H.COOH$$

the aldehyde being then estimated as iodoform.

Solutions required:

1. A one-tenth normal solution of iodine.
2. A one-tenth normal solution of sodium thiosulphate.
3. Hydrochloric acid (sp. gr. 1.018).
4. Potassium hydrate solution (56 to 1000).
5. Starch solution.

Preparation of these solutions:

1. A normal solution of iodin should contain 126.53 (molecular weight of iodin) grams of iodin in the liter, and a one-tenth normal solution, hence 12.6 grams. In order to dissolve the iodin, 25 grams of potassium iodide are dissolved in about 200 c.c. of distilled water, when the 12.6 grams of resublimed iodin are added. This solution is diluted with distilled water to the 1000 c.c. mark, and requires no further correction.

2. The one-tenth normal solution of sodium thiosulphate is prepared as described in the chapter on Acetone. (See Urine.) When treated with 1 gram of ammonium carbonate pro liter it will retain its titer almost indefinitely.

3. Preparation of the starch solution: 5 grams of starch are dissolved in 900 c.c. of water by heating, when 10 grams of zinc chloride in 100 c.c. of water are added.

METHOD.—10 to 20 c.c. of the filtered gastric juice are evaporated to a syrup after the addition of an excess of barium carbonate if free acids are present, while this is unnecessary if the Congo red test is negative. A few drops of phosphoric acid are added, the carbon dioxide driven off by boiling up once and the residue extracted, on cooling, with 100 c.c. of neutral sulphuric ether (*free from alcohol*). The layer of ether is poured off after half an hour, the ether is evaporated (no flame), the residue taken up with 45 c.c. of water, shaken, filtered, and finally treated with 5 c.c. of concentrated sulphuric acid and a pinch of manganese dioxide, in an Erlenmeyer flask. The flask is closed by a doubly perforated stopper; through one aperture a bent tube passes to a distilling apparatus, and a straight tube provided with a piece of rubber tubing, clamped off, through the other. The latter should dip well down into the liquid, and serves for passing a current of air through the solution when the distillation is completed. The mixture is distilled until about four-fifths of the contents have passed over, *excessive heat being carefully avoided*,

as otherwise the aldehyde will be decomposed into acetic acid, CO_2, and water.

To the distillate, which is best received in a high Erlenmeyer flask, well stoppered, 20 c.c. of the one-tenth normal solution of iodine are added mixed with 20 c.c. of the 5.6 per cent. solution of potassium hydrate. The mixture is shaken thoroughly and allowed to stand for a few minutes, 20 c.c. of hydrochloric acid are then added, and the excess of iodine determined by titration with the one-tenth normal solution of sodium thiosulphate. The titration is carried almost to the point of decolorization, when a little starch solution is added; the mixture is then titrated until the blue color has disappeared. The number of cubic centimeters of the one-tenth normal solution employed, viz., 20, minus the number of cubic centimeters of the one-tenth normal solution of sodium thiosulphate will indicate the number of cubic centimeters of the former required for the formation of iodoform; viz., the amount of lactic acid present in 10 or 20 c.c of gastric juice, as the case may be. As 1 c.c. of the one-tenth normal solution of iodine has been found to indicate the presence of 0.003388 gram of lactic acid, it is only necessary to multiply the number of cubic centimeters used by this figure, and the result by 10, in order to obtain the percentage.

The method described is reliable and sufficiently accurate for clinical purposes. At the same time it may be said that no more time is required than in the ordinary quantitative estimation of sugar by means of Fehling's method, or of hydrochloric acid according to the method of Martius and Lüttke.

Boas' Rapid Method.—This method is less accurate than the preceding one, but may be advantageously employed in the absence of the various reagents necessary with the former. Ten c.c. of filtered gastric juice are treated with a few drops of dilute sulphuric acid, and the albumin present removed by heat. The filtrate is evaporated to a syrup on a water bath, water added to the original amount, and this again evaporated to a small volume, fatty acids being thereby removed. The lactic acid remaining is now extracted with ether (200 c.c. for every 10 c.c. of gastric juice); the ether is evaporated, the residue taken up with water and titrated with a one-tenth normal solution of sodium hydrate, using phenolphthalein as an indicator. As 40 parts by weight of sodium hydrate (molecular weight) combine with 90 parts by weight of lactic acid (molecular weight), and as 1 c.c. of the one-tenth normal solution of sodium hydrate contains 0.004 gram of sodium hydrate, the corresponding amount of lactic acid is found from the equation: 40: 90: 0.004: x; $40x = 0.360$; $x = 0.009$. The value of 1 c.c. of the one-tenth normal solution in terms of lactic acid is thus 0.009. By multiplying the number of cubic centimeters used by this figure, the amount of lactic acid

present in 10 c.c. of gastric juice is ascertained. The result multiplied by 10 indicates the percentage.

The Fatty Acids.—Mode of Formation and Clinical Significance.—Unless much milk or carbohydrate has been ingested, fatty acids do not occur in the gastric contents under physiological conditions, and it would appear from the researches of Boas that their formation is intimately associated with that of lactic acid. After the exhibition of his test meal he was unable to demonstrate their presence either in health or in various diseases of the stomach, such as chronic gastritis atony, or dilatation referable to benign causes, etc. In carcinoma, however, fatty acids, just as lactic acid, were quite constantly found. Flügge has shown that butyric acid can be derived from lactic acid, and that this is probably its usual source.

Acetic acid fermentation presupposes the presence of alcohol, whether this is introduced into the stomach as such or whether it results from the action of yeast (Saccharomyces cerevisiæ) upon sugar. It is, hence, necessary, whenever acetic acid is met with in the gastric contents, to exclude the presence of alcohol introduced from without. Only then is it permissible to refer its presence to stagnation and decomposition of carbohydrates.

If the examination is confined to an analysis of the gastric contents obtained otherwise than after the exhibition of Boas' or Ewald's test meal, the diagnosis of pyloric stenosis with dilatation is probably always justifiable in the presence of notable quantities of butyric acid and acetic acid, while the same after a previous washing out of the stomach and the exhibition of Boas' test meal would suggest carcinoma as the cause of the stenosis.

That butyric acid may occur in the gastric contents when butter or fats in general have been ingested is, of course, not surprising, and its presence then should be looked upon as a physiological occurrence. At the same time, it should not be forgotten that butyric acid, just as lactic acid, may have been formed in the mouth, and conclusions should, hence, only be drawn when such sources of error can be definitely excluded and the amount found exceeds mere traces.

In conclusion, it may be said that in disease butyric acid is far more frequently encountered in the gastric contents than acetic acid, but the significance of the two, if alcoholism can be excluded, is the same.

Tests for Butyric Acid.—1. Butyric acid can usually be recognized by its odor alone, which is that of rancid butter. If a more definite test is desired we may proceed as follows:

2. Ten c.c. of filtered gastric juice are extracted with 50 c.c. of ether. The ether is evaporated and the residue taken up with a few cubic centimeters of water. If a trace of calcium chloride in substance is now added, the butyric acid will separate out in the form of oil droplets, the nature of which is readily recognized by the pungent

odor. If instead of adding calcium chloride a slight excess of baryta water is used, strongly refractive rhombic plates or granular, wart-like masses of barium butyrate are obtained upon evaporation.

3. Butyric acid may also be recognized by the peculiar odor of pineapple which develops when the dry residue of the ethereal solution is treated with a little sulphuric acid and alcohol. The reaction is due to the formation of ethyl butyrate (pineapple test).

Tests for Acetic Acid.—1. Like butyric acid, acetic acid can usually be recognized by its odor.

2. Ten c.c. of filtered gastric juice are extracted with ether. The ether is evaporated, the residue dissolved in a few drops of water, and neutralized with a dilute solution of sodium hydrate, sodium acetate being formed. If to this a drop or two of a very dilute solution of ferric chloride is added, a dark-red color results. With silver nitrate a precipitate is obtained which is soluble in hot water.

Quantitative Estimation of the Fatty Acids.—*Method of Cahn-Mehring, Modified by McNaught.*—The total acidity is determined in 10 c.c. of filtered gastric juice. Another 10 c.c. are evaporated to a syrup, diluted with water, and similarly titrated. The difference between the two results will indicate the amount of fatty acids present.

THE FERMENTS OF THE GASTRIC JUICE AND THEIR ZYMOGENS

Normal gastric juice contains three ferments, viz., pepsin, chymosin, and lipase.

Pepsin and Pepsinogen.—An idea of the amount of pepsin or pepsinogen in the gastric contents can only be obtained in an indirect manner, viz., by studying the rapidity with which a given amount will digest a standard quantity of albuminous material. This, however, depends to a certain extent upon the nature and concentration of the free acid present. Under normal conditions 25 c.c. of gastric juice will dissolve 0.05 to 0.06 gram of serum albumin in one hour, the same amount of coagulated egg albumin in three hours, and a like amount of fibrin in one hour and a half.

As abnormalities in the circulation and innervation of the stomach apparently do not influence the production of pepsin, or rather of its zymogen, a diminution in the degree of peptic activity, or its total absence, may be referred directly to disease of the stomach itself. The determination of the presence or absence and relative amount of pepsin in the gastric juice hence furnishes more useful information than the recognition of the presence or absence of free hydrochloric acid.

As pepsin is formed from pepsinogen through the agency of a free acid, its presence, in the absence of organic acids in notable quantities, indicates at once the presence of hydrochloric acid. It may

be said, *vice versa*, that if free hydrochloric acid is present in the gastric juice, pepsin also will be found. Should the zymogen alone be present, digestion will take place only upon the addition of an acid, while absence of digestion upon the addition of hydrochloric acid indicates the absence of both pepsin and its zymogen. At times, though rarely, a "gastric juice" is met with which is capable of digesting albumin in the absence of hydrochloric acid, owing to the presence of regurgitated pancreatic juice.

In the differential diagnosis of a chronic gastritis and a neurosis, or a dyspeptic condition referable to hyperemia of the gastric mucous membrane, the demonstration of zymogen in the absence of hydrochloric acid may, at times, be very important, bearing in mind that circulatory and nervous disturbances apparently do not influence the production of pepsinogen. An entire absence of the latter would, of course, warrant the diagnosis of anadeny of the stomach.

Tests for Pepsin and Pepsinogen.—*Test for the Enzyme.*—If the presence of free hydrochloric acid has previously been ascertained, 25 c.c. of filtered gastric juice are set aside and kept at a temperature of from 37° to 40° C., a bit of coagulated egg albumin, fibrin, or serum albumin being added. In order to permit of a comparison of results, the same amounts should always be taken; 0.05 to 0.06 gram of egg albumin, as has been shown, ought, under physiological conditions, to be digested in three hours.

Test for the Zymogen.—Should hydrochloric acid be absent, the test is made in the same manner, after the addition of from 3 to 5 drops of decinormal hydrochloric acid to 25 c.c. of the filtrate. Under such conditions usually pepsinogen alone is found.

Quantitative Estimation of Pepsin.—Accurate methods for the quantitative estimation of pepsin are unknown, and relative values only can be obtained.

Hammerschlag's Method.—Two Esbach tubes (albuminimeters) are employed. Tube *A* is filled to the mark *U* with a mixture of 10 c.c. of a 1 per cent. solution of egg albumin[1] in 0.4 per cent. of hydrochloric acid and 5 c.c. of filtered gastric juice. The second tube, *B*, receives a mixture of the same solution and 5 c.c. of water. After the tubes have been kept in the thermostat for one hour at a temperature of 37° C., Esbach's or Tsuchiya's reagent (see Urine) is added to each tube to the mark *R*. After standing for twenty-four hours the amount of precipitated albumin is read off in the two tubes. The difference indicates the amount of albumin which was digested; this raised to the square gives the corresponding amount of pepsin (which, of course, is merely relative). The method suffices for practical purposes.

Mett's Method.—Satisfactory comparative results can also be obtained with the method suggested by Mett. Capillary glass tubes

[1] The white of one egg diluted about 13 times will make a 1 per cent. solution.

are prepared measuring from 1 to 2 mm. in diameter. They are filled with white of egg, closed at the ends with bread crumbs, and coagulated in boiling water. After five minutes they are dried and the ends closed with melted paraffin. In this form they can be kept, but before use they should be examined to see that the column of albumin has not shrunk from the sides. Any bubbles that may be present disappear after two days. When needed they are cut into pieces from 1 to 2 cm. long. The length of the column digested in a given length of time serves as a measure of the digestive power of the specimen examined. In practice this column should be measured in millimeters with the aid of a magnifying glass, or a low power of the microscope, using a stage micrometer. The calculation of the corresponding amount of ferment is based upon the law of Schütz and Borrissow, viz., that the corresponding amounts of ferment in two solutions bear the same ratio toward each other as the square of the number of millimeters of the column of egg albumin which has been dissolved in the same length of time. Nirenstein and Schiff have ascertained that the length of the digested cylinder of albumin is proportionate to the length of time that digestion goes on, providing that the length of the cylinder does not exceed 7 mm. If it does exceed this, digestion proceeds more slowly. It is hence advisable in all cases to dilute the gastric juice. In this manner another difficulty also is obviated, viz., the antipeptic activity which is caused by certain substances which are normally present in solution (products of digestion, sodium chloride). Nirenstein and Schiff ascertained that a sixteenfold dilution with $\frac{n}{20}$ HCl (0.18 per cent.) is sufficient, and that this prevents the digestion of more than 3.6 mm. in twenty-four hours.

Method.—The gastric juice is obtained after giving Ewald's test breakfast. One c.c. of the filtered contents is diluted with 16 c.c. of $\frac{n}{20}$ HCl; into this solution 4 Mett's tubes are placed and the mixture is kept in the incubator for twenty-four hours. The columns of digested albumin are measured and the average ascertained; this in terms of millimeters raised to the square and multiplied by 16 (the degree of dilution) indicates the relative amount of pepsin. If the digested column measures more than 3.6 mm , the gastric juice must be diluted thirty-two times.

The unit of measure is the amount of pepsin by which 1 mm. of albumin is digested in twenty-four hours, with an acidity of 0.18 per cent. HCl.

Nirenstein and Schiff in their series found variations from 0 to 256 pepsin units.

Quantitative Estimation of Pepsinogen.—In order to estimate the amount of pepsinogen both Hammerschlag's and Mett's method can be applied after rendering the gastric contents acid with hydrochloric acid to the extent of from 1 to 2 pro mille.

The Milk-curdling Ferment and its Zymogen, viz., Chymosin (Rennin) and Chymosinogen.—Under physiological conditions chymosin and its zymogen are always present in the gastric juice. In disease the inferences that may be drawn from a quantitative estimation of the ferment and its zymogen have been formulated by Boas, to whom we are indebted for much valuable information in this connection:

1. Notwithstanding the absence of free hydrochloric acid, chymosin may be present, although in minimal traces—*i. e.*, demonstrable with a dilution of from 1 to 10 to 1 to 20 (see method below).

2. In the absence of free hydrochloric acid, the zymogen may still be present in normal amounts—*i. e.*, demonstrable with a dilution of from 1 to 100 to 1 to 150. The presence of the zymogen, especially when repeatedly observed, probably always permits of the conclusion that we are not dealing with an organic disease of the stomach, but with a neurosis or a hyperemic condition of the mucous membrane referable to disease of other organs.

3. The zymogen may occur in moderately diminished amount, 50 per cent. only being present. This is usually owing to the existence of a gastritis which has not reached its highest degree of severity. The nearer the amount of zymogen approaches the normal, the greater will be the probability of an ultimate recovery under suitable treatment.

4. The amount of the zymogen is greatly diminished (dilutions of 1 to 10 to 1 to 25 yielding a negative result) or may be absent altogether. In cases of this kind a severe and usually incurable gastritis exists, either primary or occurring secondarily to carcinoma, amyloid degeneration, etc.

5. In conditions 1, 2, and 3 the reëstablishment of the secretion of hydrochloric acid may be attempted with some prospect of success by means of stimulating remedies.

These conclusions are based upon the employment of Ewald's test breakfast, and cannot be applied to observations made after other test meals, without previous studies in this direction.

Testing for the presence of chymosin and its zymogen is of decided value in cases in which alkaline material is vomited, and where we may be called upon to decide whether this contains constituents of the gastric juice or not.

Tests for Chymosin and Chymosinogen.—*Test for the Enzyme.*—Five to 10 c.c. of milk are treated with 3 to 5 drops of the filtered gastric juice and kept at a temperature of 37° to 40° C., for ten to fifteen minutes. If coagulation occurs during this time, it may be concluded that the enzyme is present.

Test for the Zymogen.—The milk is treated with 10 c.c. of the filtered and feebly alkalinized gastric juice and with 2 or 3 c.c. of a 1 per cent. solution of calcium chloride. The mixture is kept at a

temperature of from 37° to 40° C., when in the presence of the zymogen the formation of a thick cake of casein will occur within ten to fifteen minutes.

Quantitative Estimation.—*Of the Enzyme.*—The method is based upon the fact that on gradually diluting a specimen of gastric juice a point is finally reached at which a chymosin reaction can no longer be obtained, the value being, of course, a relative one. Under physiological conditions a positive reaction can still be observed with a degree of dilution varying between 1 to 30 and 1 to 40.

The gastric juice is neutralized with a very dilute solution of sodium hydrate. Tubes are then prepared containing from 5 to 10 c.c. of the gastric juice, diluted in the proportion of 1 to 10, 1 to 20, 1 to 30, etc., to which an equal amount of neutral or amphoteric milk is added. The tubes, properly labelled, are kept at a temperature of from 37° to 40° C., and the degree of dilution noted at which coagulation still occurs.

Of the Zymogen.—The gastric juice is rendered feebly alkaline and tubes are prepared containing equal amounts of milk and gastric juice, the latter variously diluted, as above directed; the examination is then carried on in the same manner. Normally a positive reaction is obtained with a dilution varying between 1 to 150 and 1 to 100.

Lipase.—The demonstration and quantitative estimation of lipase are described in the section on the Urine. It is essential that the examination be made after a thorough washing of the stomach and the administration of a test meal which is free from fat.

ANALYSIS OF THE PRODUCTS OF ALBUMINOUS DIGESTION

In order to separate the various products of digestion from each other the following procedure may be employed:

The filtered gastric contents are carefully neutralized with a dilute solution of sodium hydrate, using litmus paper to determine the reaction; a small drop of the mixture is placed upon the paper from time to time during the addition of the sodium hydrate until no change in color is produced either on the red or the blue paper. If syntonin is present, it will be precipitated, and can be collected on a small filter. Upon the addition of an excess of dilute acid or an alkali this precipitate will again be dissolved. The filtrate is feebly acidified with dilute acetic acid, treated with an equal volume of a saturated solution of common salt, and brought to the boiling point. Any native albumin that may be present in solution is thus coagulated and can be filtered off on cooling. In the filtrate the albumoses and peptids remain.

By one-half saturation of the filtrate with ammonium sulphate,

viz., by adding an equal amount of a saturated solution of ammonium sulphate, the primary albumoses can be precipitated. If then the neutral filtrate is treated with one-half its volume of a saturated solution of ammonium sulphate, which will thus give a two-thirds total saturation, a portion of the deutero-albumoses (fraction *A*) separates out on standing. This is filtered off and the solution saturated with ammonium sulphate in substance; the deutero-fraction *B* is thrown down, and on acidifying the filtrate with one-tenth of its volume of a solution of sulphuric acid that has been saturated with ammonium sulphate, and of which 10 c.c. correspond in strength to 17 c.c. of a $\frac{n}{10}$ solution of sodium hydrate, the last traces of deutero-albumoses (fraction *C*) will separate out on standing.

The filtrate contains the "peptones." To demonstrate these a 2 per cent. solution of cupric sulphate is added drop by drop, when in the presence of peptones a rose- to a purplish-red color will develop.

TESTS FOR THE PRODUCTS OF CARBOHYDRATE DIGESTION

Starch may be recognized by the fact that it strikes a blue color with a solution of iodopotassic iodide, while the same solution gives a violet or mahogany brown with erythrodextrin. To this end it is only necessary to add a drop or two of Lugol's solution to a few cubic centimeters of the filtered gastric juice. The presence of achroödextrin may be inferred if no change in color occurs upon the addi ion of the reagent.

Maltose and dextrose, which both react with Fehling's solution and undergo fermentation, differ from each other in the fact that the former does not reduce *Barfoed's reagent* on boiling. This is prepared by adding 1 per cent. of acetic acid to a 0.5 to 4 per cent. solution of cupric acetate. The rotary power of maltose is about three times as strong as that of dextrose: (a) D = 150.4, as compared with 52.5.

GASES

The stomach always contains a certain quantity of gases which have partly been swallowed and partly have passed into the stomach from the duodenum. As fermentative processes in health occur only when carbohydrates or fats have been ingested, and then only to a slight degree, nitrogen, oxygen, and carbon dioxide are the only gases found during the process of albuminous digestion. As the oxygen swallowed is, moreover, largely absorbed by the blood, and two volumes of carbon dioxide are returned for one volume of oxygen, the presence of large amounts of the former and small amounts

of the latter is readily explained. In an analysis of the gases contained in the stomach of a dog which had been fed on meat, Planer found the following proportions:

Carbon dioxide	25.2	vol. per cent.
Oxygen	6.1	" "
Nitrogen	68.7	" "

With a strict vegetable diet, on the other hand, hydrogen may also be found (Planer):

	Man.		Dog.	
Carbon dioxide	20.79	33.83	32.9	vol. per cent.
Oxygen		0.37	0.8	" "
Nitrogen	72.50	38.22	66.3	" "
Hydrogen	6.71	27.58		

Marsh gas, CH_4, a product of the fermentation of cellulose, may also be found in pathological conditions, but it is as yet an open question whether marsh gas is formed in the stomach or passes into the stomach from the small intestine. Such observations must, however, be regarded as rarities. In one case examined by Ewald and Ruppstein, in which alcohol, acetic acid, lactic acid, and butyric acid were found in the vomited material, an analysis of the gases gave the following result:

Carbon dioxide	20.6	vol. per cent.
Oxygen	6.5	" "
Nitrogen	41.4	" "
Hydrogen	20.6	" "
Marsh gas	10.8	" "

Traces of olefiant gas and of hydrogen sulphide were also found. It is curious to note that in this case the patient, who, according to his own statement, had a "vinegar factory in his stomach on one day and gas works on another day," was occasionally able to light the eructated gas at the end of a cigar-holder, where it burnt with a faintly luminous flame. McNaught has reported a similar case in which the analysis furnished the following results: Carbon dioxide, 56 per cent.; hydrogen, 28 per cent.; marsh gas, 6.8 per cent.; atmospheric air, 9.2 per cent.

Ammonia and hydrogen sulphide are also at times met with; their presence is always due to albuminous putrefaction.

Boas found that hydrogen sulphide is quite commonly present in cases of dilatation referable to benign causes, while it is almost always absent in carcinoma. He adds that it is never found when lactic acid is present. In acute gastritis it may be observed temporarily. In a number of cases of carcinoma I have never found hydrogen sulphide. In one case reported by Strauss the Bacillus coli communis was apparently concerned in its production.

To obtain a knowledge of the gases formed in the stomach during the process of digestion it is only necessary to fill an ordinary Doremus ureometer, or an Einhorn saccharimeter, with the unfiltered gastric contents, and to keep it at a temperature of from 37° to 40° C., when the evolution of gas can be followed closely and the necessary tests made. The presence of carbon dioxide is readily recognized by passing a small amount of sodium hydrate, in concentrated solution or in substance, into the tube, after the evolution has entirely ceased, when the fluid will rise. If other gases are present at the same time, they will remain after the carbon dioxide has been absorbed. Hydrogen sulphide is readily recognized by its odor and by the fact that it will color a piece of filter paper, moistened with a few drops of sodium hydrate and lead acetate, a more or less pronounced brown or black. The test is conveniently made by filling a test-tube about half-full with the gastric contents and closing it with a cork stopper to which a strip of lead paper, prepared as indicated, is fastened.

Marsh gas is recognized by the fact that it burns with a scarcely luminous flame.

The eructation of gas formed in the stomach should not be confounded with the so-called *eructatio nervosa*, in which no gas is either eructated or air simply enters the esophagus and is expelled again with a loud, explosive noise. This may frequently be observed in neurasthenic and hysterical individuals, and is to a greater or less degree under the control of the will.

ACETONE

The presence of acetone in the gastric contents in pathological conditions has repeatedly been observed, especially by v. Jaksch and Lorenz, and it is curious to note that the latter was at times able to demonstrate larger quantities of the substance in the gastric contents than in the urine.

In the *primary* diseases of the gastro-intestinal tract acetone was met with quite constantly, while it was observed but rarely in the secondary forms, and never in the gastric neuroses. This, however, is denied by Sovelieff, who claims to have found traces of acetone in one case of nervous dyspepsia, while negative results were obtained in all other diseases of the stomach.

In order to test for acetone, the gastric contents are distilled after the previous addition of a small amount of phosphoric acid (1 to 1000), when the tests of Reynolds and Gunning (see Urine) are applied to the distillate. If both reactions furnish a positive result the presence of acetone may be regarded as demonstrated. Dennigès' test may also be employed, and can be applied to the filtered contents directly. (See Urine.)

VOMITED MATERIAL

Food Material.—The vomiting of large amounts of totally undigested meat two or three hours after its ingestion is met with only in conditions associated with an entire absence of digestive juices from the stomach—*i. e.*, in cases of atrophic cirrhosis of the stomach (anadeny of Ewald). This condition is not to be confounded with the regurgitation of undigested food, mixed with mucus and saliva, which is seen in cases of stricture of the esophagus or of the cardiac orifice of the stomach. While at the outset of the latter condi-

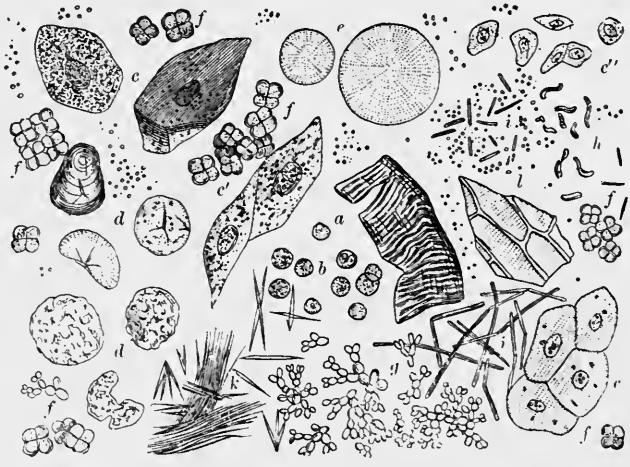


FIG. 70.—Collective view of vomited matter. (Eye-piece III, objective 8 A, Reichert.) *a*, muscle fibers; *b*, white blood corpuscles; *c*, *c'*, squamous epithelium; *c''*, columnar epithelium; *d*, starch grains, mostly changed by the action of the digestive juices; *e*, fat globules; *f*, sarcinæ ventriculi; *g*, yeast fungi; *h*, forms resembling the comma bacillus found by the author once in the vomit of intestinal obstruction; *i*, various microörganisms, such as bacilli and micrococci; *k*, fat needles, between them connective tissue derived from the food; *l*, vegetable cells. (v. Jaksch.)

tion the regurgitation of food occurs immediately, or at least very soon, after a meal, it may take place between meals in the later stages of the disease when dilatation has occurred. The recognition of the origin of the material brought up may then be exceedingly difficult. In such cases an examination should be made for biliary coloring matter, which, if present, will, of course, immediately exclude the esophagus as the source of the material ejected. Unfortunately, however, the reverse does not hold good. Small amounts of undigested meat are of no significance. The vomiting of well-digested food is observed in some of the neuroses of the stomach, and also in certain cases of acute and subacute gastritis, ulcer of the stomach, and chronic gastritis in its early stages. The vomiting

referable to cerebral and spinal diseases also belongs to this category. In this connection it is very important to inquire into the existence of nausea previous to the vomiting, for, as is well known, considerable amounts of saliva and mucus may be swallowed if much nausea has existed, the result being that the process of digestion is arrested before the occurrence of vomiting. In such an event it would be erroneous to conclude that, because the material ingested has not reached that stage of digestion which would be expected at the time of the vomiting, the stomach is incapable of properly performing its functions.

Mucus.—The constant presence of large amounts of mucus in the gastric contents obtained with the stomach tube is almost pathognomonic of the mucous form of gastritis, while its presence in vomited matter may be referable to preëxisting nausea and temporarily increased production. In cases of pharyngitis moderate amounts of mucus are frequently found. The vomiting of pure mucus, according to Boas, is always pathognomonic of the absence of dilatation of the stomach, a statement founded on reason, as it is altogether unlikely that no particles of food should be brought up at the same time.

Under the term *gastrosuccorrhea mucosa,* Dauber has described a condition in which large amounts of mucus are secreted by the non-digesting organ, in the absence of symptoms pointing to a gastritis. I have observed a similar case occurring in a neurasthenic patient, in which enormous quantities of mucus could at times be obtained from the fasting organ, but never during the process of digestion. A mild degree of hyperchlorhydria existed at the same time, as well as enteritis mucosa and rhinitis mucosa. The motor power was practically normal.

Mucus is readily recognized on simple inspection by its glossy appearance. Chemically, it is distinguished by its behavior toward acetic acid (see Urine).

Saliva.—The vomiting of pure saliva in the morning upon rising is a common symptom of chronic pharyngitis, which in turn frequently carries in its train a chronic gastritis; it constitutes the so-called *vomitus matutinus* of alcoholics. Saliva, like mucus, is, of course, always present in the gastric contents in small amounts. Larger amounts are usually referable to an increased secretion owing to the existence of nausea. Chemically, saliva is best recognized by testing for the presence of the sulphocyanides. (See Saliva.)

Bile.—Bile is rarely observed in the gastric contents brought up by the stomach tube, but is frequently seen in vomited matter, of which it may be said to be a constant constituent whenever the vomiting has been intense or frequently repeated. Its presence in the former case should always excite suspicion of the existence of stenosis of the descending or horizontal portion of the duodenum or

the beginning of the jejunum. This diagnosis becomes the more probable the more constant its presence.

Pancreatic Juice.—Mixed with the bile there is probably always some pancreatic juice, and it has been suggested that the constant absence of this constituent, in the presence of bile, is strongly suggestive of pancreatic disease or of obstruction of the pancreatic duct (the ductus Wirsungianus).

The demonstration of pancreatic juice in the stomach is possible only if the reaction is neutral or alkaline, as the pancreatic trypsin is destroyed by pepsin-hydrochloric acid. If then hydrochloric acid is absent it is well to insure a distinctly alkaline reaction by adding a little 1 per cent. solution of sodium carbonate; a flake of fibrin is added and the mixture placed in the incubator; if digestion takes place the presence of trypsin is established. The flakes of fibrin may be previously colored with a little Magdala red; as digestion takes place the red is liberated and colors the fluid.

Blood.—The presence of unaltered blood in the gastric contents is usually recognized without difficulty. If the hemorrhage has taken place in the stomach the color usually is dark brown or black owing to the action of the gastric juice upon the hemoglobin. Blood that is bright red in color and frothy is generally referable to a pulmonary hemorrhage, but it may happen that such blood is swallowed and remains in the stomach for some time and may then also appear brown or black. In the event of a large gastric hemorrhage, on the other hand, the color of the vomited blood may be bright red.

In order to recognize mere traces when the macroscopic and even the microscopic examination do not point to the presence of blood, anyone of the tests for occult blood may be applied (see Feces).

Hemorrhage from the stomach may be observed in the most diverse conditions. It is either dependent upon a primary disease of the organ, such as ulcer and carcinoma, or it occurs secondarily to disease of other organs, leading to a hyperemic condition of the gastric mucosa, such as the various forms of cardiac, renal, and hepatic disease, in connection with menstrual abnormalities, etc. In melena, purpura hæmorrhagica, pernicious anemia, etc., the cause of the hemorrhage cannot always be determined. Nervous influences also may take part in the causation of gastric hemorrhage.

Pus.—The occurrence of pus in vomited matter, referable to disease of the stomach itself, is uncommon. It is seen practically only in cases of phlegmonous and diphtheritic gastritis, and, as Strauss has pointed out, in carcinoma affecting the smaller curvature and the region of the fundus. In such cases it is not uncommon to obtain as much as one-half to two tablespoonfuls of a mucopurulent fluid from the non-digesting organ. As the motor function in this form of carcinoma is often unimpaired, the symptom may be

of value in diagnosis. The presence of larger quantities usually indicates perforation into the stomach of an accumulation of pus from a neighboring organ. An abscess of the liver, a suppurative pancreatitis, an abscess of the colon, or a subphrenic abscess may prove to be its primary source. When present in considerable amount, pus is, of course, readily detected with the naked eye; if any doubt should arise, a microscopic examination will determine the question.

Stercoraceous Material.—Very important from a clinical standpoint is the vomiting of stercoraceous matter which is notably observed in cases of ileus. Usually this is recognized without difficulty by its odor, which is referable to the presence of skatol. If any doubt should arise, it is only necessary to distil the vomited matter after the addition of a little phosphoric acid, and to test for the presence of phenol, indol, and skatol in the distillate, as described in the chapter on Feces. When chiefly derived from the small intestine, the vomited matter, according to v. Jaksch, will contain bile acids and bile pigment together with an abundance of fat, which may be detected by chemical or microscopic examination. The reaction is usually alkaline or feebly acid.

Parasites.—Of parasites, ascarides, segments of teniæ, trichinæ, Ankylostoma duodenale, and Oxyuris vermicularis are, at times, encountered. Protozoa have been described in the stomach contents of patients with carcinoma by Hensen, Strübe, Zabel, Ullmann, Cohnheim, Nichols and others. (See Microscopic Examination of Stomach Contents.)

Odor.—The odor of normal gastric juice is peculiar, suggesting the presence of an acid, which can be sharply distinguished from acetic or butyric acid. If blood is present in large amount, the vomitus emits an odor which is perfectly characteristic. A feculent odor is met with in cases of enterostenosis or in the presence of an abnormal communication between the stomach and the small or large intestine. A putrid odor may be observed in cases of ulcerative carcinoma, pyloric stenosis referable to ulcer, simple carcinoma of the stomach, muscular hypertrophy of the pylorus, stenosis due to inflammatory adhesions, etc. In cases of phosphorus poisoning the vomited matter emits an odor of garlic; the odor observed in uremic conditions is referable to ammonia; a carbolic acid odor is met with in cases of poisoning with this substance.

MICROSCOPIC EXAMINATION OF THE GASTRIC CONTENTS

If gastric juice is allowed to stand, small tapioca-like bodies will collect at the bottom of the vessel, which upon microscopic examination will be seen to contain numerous snail-shell-like formations, occurring either singly or collected in groups. These probably consist of altered mucin, as they can be produced artificially by adding

a sufficient amount of dilute hydrochloric acid to saliva. According to Boas, they are of no diagnostic significance.

Epithelial cells, fragments of the epithelial lining of the ducts of glands, as well as goblet cells, are not infrequently met with in the juice obtained from the non-digesting organ. In addition, various microörganisms, such as the Leptothrix buccalis, Bacillus subtilis, saccharomyces, micrococci (often arranged in the form of tetrahedra), Clostridium butyricum, etc., may be encountered.

Among the bacteria which may be found in the gastric contents under pathological conditions the bacillus described by Boas and Oppler is undoubtedly the most important, and has attracted much attention. It is quite constantly present in carcinoma, at a time when lactic acid can be demonstrated in large amount. It is an active lactic acid producer and its presence may hence be regarded as indicating advanced lactic acid fermentation. It is almost always absent in non-malignant disease of the stomach. The organism (Fig. 71) is non-motile, and essentially characterized by its great length and by the fact that the individual bacilli are frequently seen joined end to end; forming long threads and zigzag lines. Occasionally true dichotomy may be observed. Often the entire field of vision is filled with dense conglomerations, and in advanced cases it is usual to find the Boas-Oppler bacillus present almost exclusively. The organism is readily stained with the usual anilin dyes. I have succeeded in growing it on blood serum and usually also on plain agar, but it is very apt to undergo changes in size which may lead one to think that it has been lost or overgrown by other bacilli. Growth may sometimes be obtained by rendering the culture medium acid with lactic acid to the extent to which this was present in the stomach contents. Galt and Iles are believed to have demonstrated the identity of the organism in question with the B. bulgaricus.

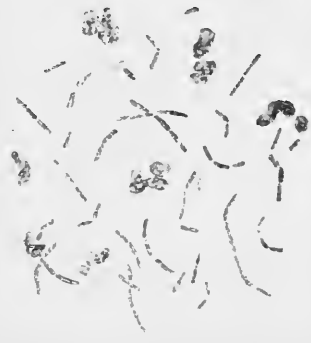


Fig. 71.—Boas-Oppler bacillus.

Tubercle bacilli may be found in vomited matter in cases of phthisis, where the sputum has been swallowed. Tubercular ulceration of the stomach is exceedingly rare. Simmonds reports that in 2000 autopsies of tubercular individuals the condition was noted only eight times.

Sarcinæ (Fig. 70) occur in the form of peculiar colonies of cocci, arranged in squares or tetrahedra, resembling cotton bales. Not infrequently they are encountered under normal conditions, but only

in small numbers. In pathological conditions, on the other hand, a drop of the gastric contents may constitute an almost pure culture. A case is on record in which the pylorus had become entirely occluded by an inspissated mass of these organisms. It is curious to note that in advanced cases of carcinoma of the stomach sarcinæ are practically never seen, although the conditions are apparently most favorable for their development. Oppler was unable to find them twenty-four hours after their introduction in large numbers and in pure culture. In cases of carcinoma of the curvatures and the walls, as also in advanced pyloric carcinoma, sarcinæ were never found, while they may be present in incipient cases of pyloric carcinoma so long as hydrochloric acid is secreted.

Protozoa have been found in the stomach contents by several observers. Nichols has collected 23 cases from the literature. The most common are trichomonads and next in order Megastoma entericum (Lamblia intestinalis); whether or not still other varieties occur is not clear from the meager descriptions which are usually given. Flagellates, amebas, and monads are mentioned in a general way. Megastoma and trichomonads may be found together. The presence of protozoa is most common in carcinoma of the stomach (19 out of 23 cases). The reaction of the material in which they are found is almost invariably alkaline or neutral. It is noteworthy that in several cases trichomonads were also found in carious teeth and in many in the stools of the patients.

In esophageal carcinoma protozoa have also been found in the esophageal material.

From the available data there can be no question that the presence of protozoa in the stomach contents is suggestive of non-obstructive carcinoma. To hunt for the parasites it is best to obtain material from the fasting organ and to examine this as soon as possible, taking care that it is not exposed to cold. Attention should be especially directed to any solid particles that may be visible with the naked eye.

In vomited material containing biliary coloring matter, leucin, tyrosin, and cholesterin are quite commonly observed, and may be recognized by the form of their crystals, as well as by their chemical reactions, which are described elsewhere.

The occurrence of blood and pus in the gastric contents has been considered.

It not infrequently happens that small *shreds of mucous membrane* are brought away by the stomach tube, and in cases of chronic gastritis, hyperchlorhydria not dependent upon ulcer, and in some of the neuroses this is, indeed, not at all uncommon. Boas even suggests that in the neuroses, where fragments of mucous membrane are so readily detached, this may possibly be connected etiologically with the formation of ulcers, and there can be no doubt that the mere action of the abdominal muscles exerted during the process

of defecation may be sufficient to detach such fragments. From the microscopic appearance of the particles the diagnosis between a gastric neurosis and one of the various forms of chronic gastritis may sometimes be made, and the same may be said to hold good in the differential diagnosis between a true gastritis and a glandular insufficiency referable to passive congestion of the gastric mucosa.

At times *tumor particles* also are found in the gastric contents; they should be hardened at once, and then sectioned.

EXAMINATION OF THE MOTOR POWER OF THE STOMACH

Under physiological conditions the stomach should contain but few particles of food, or none at all, six hours after the ingestion of Riegel's meal, or one and one-half to one and three-quarters hours after that of Ewald. A delay in the propulsion of the gastric contents may be referable to the existence of a simple atony or to dilatation of the stomach. According to Boas, an atony may usually be diagnosticated if, following the exhibition of a supper consisting of bread and butter, cold meat, and a large cupful of tea, the stomach is found empty in the morning, providing, of course, that symptoms exist which point to atony or dilatation. It should be remembered, however, that in cases of acute and subacute gastritis, in the absence of a more serious lesion, food may be found in the stomach twenty-four hours after its ingestion. A dilatation may, on the other hand, be diagnosticated if the stomach under the same conditions contains a considerable amount of food. In such cases it happens that not only remnants of the test supper, but remains of meals taken one, two, three, or even more days previously are found. The quantities, moreover, which may be obtained at the time of examination are often surprisingly great, and may amount to sixteen pounds or more. Portel cites the case of the Duc de Chausnes, one of Paris' greatest gourmands, whose stomach could hold 4.5 liters—*i. e.*, 8 pints.

The following methods may be employed for the purpose of testing the motor power of the stomach:

Leube's Method.—Six hours after the ingestion of Riegel's meal the stomach is washed out with about 1000 c.c. of water. In the presence of only slight traces of food the motor power may be regarded as normal. This method is undoubtedly the most convenient for practical purposes.

The Salol Test of Ewald and Sievers.—This test is based upon the observation that salol is decomposed into phenol and salicylic acid only in an alkaline medium. As the salicylic acid is eliminated in the urine as salicyluric acid, it is possible to determine the time of the passage of the salol from the stomach to the small intestine.

A capsule containing 1 gram of salol is given to the patient immediately after his breakfast or dinner, when separate portions of

urine passed one-half, one hour, two hours, and twenty-four hours later are tested by adding a small amount of a solution of ferric chloride. In the presence of salicyluric acid a violet color results. Under normal conditions a positive reaction is obtained after from forty-five to seventy-five minutes. A further delay may usually be regarded as indicating the existence of motor insufficiency. If no result is obtained after twenty-four hours, a pyloric stenosis undoubtedly exists. Under normal conditions, furthermore, it will be observed that the salol elimination is completed after twenty-four hours, while in cases of dilatation of the stomach a positive reaction may still be obtained after thirty hours. It is thus possible to distinguish between dilatation and descent of the stomach.

The test, while it is convenient and usually yields fair results, is not altogether reliable, as the decomposition of the salol may at times occur in the stomach, owing to the presence of alkaline mucus, or may be delayed in the intestines owing, to the existence of acid fermentation, etc.

EXAMINATION OF THE RESORPTIVE POWER OF THE STOMACH

To this end a capsule containing 0.2 gram of potassium iodide is given to the patient shortly before a meal, and the saliva examined for the presence of potassium iodide at intervals of from two to three minutes. To this end strips of filter paper moistened with starch solution are immersed in the saliva, which has been acidified with nitric acid; the paper turns blue if iodide be present. Under normal conditions a violet color is obtained after from six and one-half to eleven minutes, and a bluish tint after from seven and one-half to fifteen minutes. In pathological conditions a delayed reaction is observed in almost all diseases of the stomach, and is especially marked in cases of dilatation and carcinoma, less so in chronic gastritis, and variable in ulcer.

Absolute conclusions, however, cannot be drawn from results thus obtained, as a normal reaction time has also been observed in cases of dilatation and chronic gastritis.

INDIRECT EXAMINATION OF THE GASTRIC JUICE

Günzburg's Method.—In those cases in which for any reason the introduction of the stomach tube is contraindicated or impracticable the following method, suggested by Günzburg, may be employed:

A tablet of 0.2 to 0.3 gram of potassium iodide is inserted into a piece of the thinnest possible, strongly vulcanized rubber tubing, measuring about 2.5 cm. in length. The ends are folded as shown in Fig. 72, and the little package tied with three threads of fibrin

hardened in alcohol. Every package should be examined before use, by immersion in warm water for several hours, to determine its tightness, testing for the presence of potassium iodide by means of starch paper and fuming nitric acid. One of these packages is swallowed by the patient three-quarters to one hour after an Ewald test breakfast, and the saliva tested for potassium iodide at intervals of fifteen minutes, until a positive result is reached or until six hours have elapsed. It is unnecessary to wait longer than six hours. In the presence of free hydrochloric acid the threads of fibrin are dissolved and the potassium iodide absorbed. Under normal conditions a positive reaction is obtained after from one to one and three-quarters hours, while anachlorhydria undoubtedly exists if no result is obtained within five or six hours. In cases of hypochlorhydria the reaction is delayed for more than two to three hours. Günzburg further advises that the resorption test with potassium iodide be also made, and that the reaction time be deducted from that taken up in the elimination of the iodide contained in the package. Several tests, moreover, should be made in the same case.

FIG. 72.—A fibrin-potassium-iodide package of Günzburg.

I have had occasion to experiment with packages obtained from Germany, and manufactured according to the directions of Günzburg.[1] In most of the packages the threads of fibrin had become brittle and were broken in transit. The results obtained with about twenty intact specimens, however, were entirely satisfactory, and it is to be regretted that the packages cannot be obtained in the American market.

Similar packages have been constructed by Sahli (*desmoid reaction*). In this case pills of methylene blue or iodoform are inclosed in little pieces of rubber tissue and closed with catgut. They are administered at the noon meal and the urine (viz., saliva) tested at 5 and 7 P.M. and again in the morning.

Reach has of late made use of barium iodate and the oxyiodate of bismuth for the same purpose, but without inclosing the substance in rubber. As hydrochloric acid only is capable of liberating the iodine from these bodies, they may be employed instead of the Günzburg packages. As a result of his examinations, he concludes that in the presence of hydrochloric acid iodine can thus be demonstrated in the saliva within eighty minutes. He finds, however, that at times the reaction occurs later than might have been supposed from the amount of hydrochloric acid found.

[1] Göthe Apotheke, Frankfurt a. M.

CHAPTER IV

THE FECES

The feces constitute a mixture of indigestible and undigested particles of food, of unabsorbed secretions of the gastro-intestinal tract, and their decomposition products, together with intestinal mucus, epithelial cells, and bacteria.

GENERAL EXAMINATION OF THE FECES

General Characteristics.—Number of Stools.—The number of stools which may be passed in the twenty-four hours is subject to wide variation, even under physiological conditions, but is usually constant for one and the same individual. One or two stools *pro die* may be regarded as normal. Exceptions, however, are frequent. Persons are thus met with who have but one stool every two to four days, and cases are on record in which only one passage occurred every seven to fourteen days, the individuals evidently enjoying perfect health. On the other hand, the number of stools may be increased to three or four under strictly normal conditions. *Hence the importance of accurately ascertaining the habitual number of stools in every individual.* It would thus be manifestly wrong to regard the passage of three stools daily as diarrhea, or the passage of only one stool in forty-eight hours as constipation, if this number has been habitual throughout life.

Diarrhea is said to exist when the consistence of the stools is materially diminished; the number is then also usually increased. This may vary from two to thirty, forty, and even fifty in the twenty-four hours. On the other hand, a single stool in the twenty-four hours may constitute diarrhea. The most extreme grades of diarrhea are observed in Asiatic cholera, dysentery, and the summer diarrhea of infants.

Amount.—In those cases in which more than one or two stools occur in twenty-four hours it is well to ascertain the amount actually passed. The normal amount varies between 100 and 200 grams. This quantity is increased by a diet rich in vegetable and starchy foods, and is diminished by one rich in animal proteins, so that 60 and 270 grams may be regarded as the extreme limits in health. Such amounts as 500 and 1000 grams are certainly abnormal.

Average quantities for various ages are given in the following table, which is taken from Schmidt and Strassburger:

Age.	Diet.	Average amount of feces in twenty-four hours.
Child, 1 month old	Mother's milk	3.3 grams
" 2 to 3 months old	" "	6.5 "
" 7 " "	Variable	15 to 56 "
" 9 " "	Cow's milk with additions	59.0 "
" ¾ to 2 years old	Mixed	77.0 "
" 4 " "	"	101.0 "
" 6 " "	"	134.0 "
" 9 " "	"	117.0 "
" 11 " "	"	138.0 "
Adult	"	131.0 "

Unusually large amounts of fecal matter may be observed following an attack of constipation of long duration or an attack of obstruction. Lynch reports a remarkable instance in which, following a prolonged attack of constipation, an enema caused the evacuation of 20 kg. of fecal matter. Especially large amounts of feces are observed in cases of biliary obstruction, where 1100 grams may be exceeded. In cases of "fermentative dyspepsia" the amount may also be large, varying between 400 and 900 grams, while the patients are on a diet on which normal individuals would pass from 200 to 270 grams in the twenty-four hours. Still larger amounts are noted in cases of enteritis. Schmidt mentions a case in which 2780 grams were eliminated (these figures have reference to a three days' experiment with a test diet; see p. 240). The average weight of *dried* feces in normal individuals while on Schmidt's test-diet is about 54 grams (varying between 45 and 62 grams) in the three days of the test-period. The average in five cases of "fermentative" dyspepsia, studied by Schmidt was 127.4, the average in "gastrogenous" diarrhea with achylia gastrica, 98.9 grams. His highest figures are noted in obstruction of the common duct, with 175.6 grams as average and 215.4 as maximum. In one case of obstructive jaundice associated with carcinoma of the pancreas, Pratt found 419 grams as weight of one metabolism period of three days and 355 grams in another. The same writer mentions a case of chronic fatty diarrhea and glucosuria without jaundice where the feces weighed 438 grams.

Consistence and Form.—The consistence of a stool depends essentially upon the amount of water present, and hence upon the nature of the food ingested, being softer with a purely vegetable diet (80 to 85 per cent. of water) than with a diet rich in animal proteins (60 to 65 per cent.). With a mixed diet the amount of water corresponds to about 75 per cent. As a general rule, normal stools exhibit the characteristic cylindrical form and are fairly firm. Mushy stools, however, are also seen quite frequently, and round, scybalous masses, although far more common in constipation, may likewise be observed in health. The individual scybala usually vary in size

from that of a hazelnut to that of a walnut, and are frequently provided with one or two indentations which represent impressions of the tenia of the colon. Still smaller masses, resembling the dejecta of sheep, may also be seen. Their presence was formerly regarded as characteristic of stricture of the colon, but they are likewise found in ordinary cases of chronic constipation. Fecal ribbons and columns of the diameter of a pencil are found in cases of enterospasm of neurotic origin, as well as in stricture of the colon.

Odor.—The repugnant odor of the feces is, to a large extent, due to the presence of indol and skatol and in some cases also to hydrogen sulphide, methane, and phosphine. A most disagreeable odor is met with in the so-called acholic stools. The odor of fatty acids is observed in the lighter grades of infantile diarrhea, while a markedly putrid odor is associated with its severer forms. A very characteristic, sperm-like odor is noted in the stools of cholera, owing to the presence of considerable quantities of cadaverin. A truly rotten stench is present in the gangrenous form of dysentery, and in carcinomatous and syphilitic ulceration of the rectum. An ammoniacal odor is due to an admixture of urine undergoing ammoniacal decomposition.

Color.—The color of the feces varies, according to the nature of the food ingested from a light to almost a blackish brown, a firm stool being in general darker than a thin stool. A stool that has remained exposed to the air is also somewhat darker upon its outer surface than in its interior, owing to processes of oxidation. In nursing infants, in consequence of the exclusive ingestion of milk, the color is light yellow.

Under normal conditions the color is never due to native biliary coloring matter, but is largely dependent upon the presence of urobilin. It is, furthermore, influenced by the nature of the food, chlorophyll tending to produce a greenish color, starches a yellowish tinge. If much blood is present in the food the feces may be almost black, owing to the formation of hematin. Huckleberries and red wine likewise produce a blackish color, chocolate and cocoa a gray; preparations of iron, manganese, and bismuth color the feces dark brown or black, owing to the formation of sulphides of these metals; the green color of calomel stools was formerly supposed to be due to the formation of a sulphide, but is more likely caused by the presence of biliverdin. Santonin, rheum, and senna produce a yellow color. Quite characteristic also are the ipecacuanha stools, which closely resemble the so-called acholic stools.

The color of the feces in disease may vary a great deal. When unaltered bile is present, the stools may assume a golden-yellow, a greenish-yellow, or even a green color. In cases of biliary obstruction or suppression, on the other hand, they become pasty and have a grayish or even a white color. This, however, is not so much due to the absence of coloring matter derived from the bile as to

an insufficient absorption of fats, as was shown by Strümpell, who succeeded in obtaining stools of a light brown color after feeding patients affected with catarrhal jaundice upon a diet containing minimal amounts of fat. *Such acholic or colorless stools*, as it would be better to say, are not only found associated with biliary obstruction, but may also occur when the ducts are patent. They have been observed in various cases of leukemia, carcinoma of the stomach or intestine, in simple infantile enteritis, chronic nephritis, chlorosis, scarlatina, tubercular enteritis, and especially frequently in debilitated consumptives and in cases of chronic tubercular peritonitis in children. In some of these conditions, as in tuberculosis of the intestines and of the peritoneum, the lack of color is probably due to a diminished absorption of fats. In others, however, this explanation does not hold good, as abnormally large amounts of fat are not necessarily present. In such cases the lack of color is probably referable to the formation of colorless decomposition products of bilirubin, such as the leuko-urobilin of Nencki. In this connection it may be interesting to note that in those cases in which the biliary ducts are patent the color of the stools may vary not only from day to day, but even within the twenty-four hours. A neurasthenic patient occurring in my practice thus passed an acholic stool almost every morning and usually colored feces in the afternoon, for a period of several weeks.

Generally speaking, the color of the stools becomes lighter the larger the number of movements, and *vice versa*. In Asiatic cholera and dysentery they may be colorless, while in severe constipation the scybalous masses are almost black.

An admixture of *pus* in notable amounts also gives rise to a characteristic color, as is seen in cases of dysentery, syphilitic and carcinomatous ulceration of the colon and rectum, following the perforation of a parametritic or periproctitic abscess into the rectum, etc.

Carter and MacMunn have pointed out that at times a chromogen may be present in the feces, which on exposure to the air is transformed into a red pigment, simulating blood-coloring matter. They report three cases in which this was observed. MacMunn expresses the opinion that the substance in question is closely related to stercobilin. The stools showed streaks of red upon the surface, and after further exposure and repeated agitation turned a pronounced blood red throughout.

Green stools are observed especially in infants, and may be referable to two different causes, being dependent, on the one hand, upon the presence of a bacillus, described by Le Sage, which produces a green coloring matter, while on the other it may be referable to biliverdin. When green stools occur frequently, this condition is associated with the clinical symptoms of a severe cholera infantum. Such stools have also been noted in dysentery referable to infection with the Bacillus pyocyaneus.

If *blood* is present the stools may present a scarlet red, a dirty, brownish red, a coffee, or even a perfectly black color. *Adherent blood,* usually bright red in color and found on scybalous masses, is probably always derived from the rectum or anus, while a change in color, indicating an earlier date of the bleeding, usually points to the colon.

An *intimate admixture of blood* to the stool, the color being at the same time altered, so as to vary from a brownish red to black (owing to the presence of ferrous sulphide), is indicative of hemorrhage into the stomach or the small intestine. The darker the color the more remote from the anus will be, as a rule, the seat of the hemorrhage. Black or coffee-colored stools are thus observed in cases of ulcer of the stomach or of the duodenum, in melæna neonatorum, and similar conditions.

When profuse intestinal hemorrhages take place, however, as in some cases of typhoid fever and melena, and particularly when diarrhea exists at the same time, the blood which appears in the stools may be changed very little or not at all.

While simple inspection or a microscopic examination of the feces will often determine whether or not blood is present, it has been ascertained that *occult* bleeding may frequently occur where the presence of blood can only be established by special chemical examination. Evidence of such occult bleeding can be obtained in malignant growths involving the gastro-intestinal tract, in ulcer (over 80 per cent. of the cases), hemorrhagic pancreatitis, catarrhal jaundice (at the height of the disease), general venous stasis referable to heart lesion. Other sources of bleeding must, of course, be excluded, and the diet during the period of examination should be free from meats.

Tests for Occult Blood.—To test for occult blood any one of the four tests described below may be employed:

The Phenolphthalein Test.—The reagent is prepared as follows: 100 c.c. of a 20 per cent. solution of caustic alkali (NaOH) are treated with 2 grams of phenolphthalein and an excess (20 grams or more) of zinc dust. The bright rose-colored solution is heated gradually until it has become decolorized or rather until it has assumed a slightly yellowish tone, owing to a reduction of the phenolphthalein to phenolphthalin. The supernatant fluid is poured off into a colored glass bottle and the access of air prevented by the addition of a little liquid paraffin (20 to 30 grams), which floats upon the top. On adding 1 c.c. or so of this reagent to a solution of a small bit of the suspected fecal matter in water (about 2 c.c.), and treating with one or, at most, two drops of a 10 per cent. solution of hydrogen peroxide, a bright red color will develop, owing to a reoxidation of the phenolphthalin to phenolphthalein through the agency of the oxidase of the blood in the presence of the peroxide. The reaction is exceedingly delicate, indicating the presence of blood in a dilution of 1 to 800,000 (*i. e.*, 0.000012 per cent.).

Aloin Test.—If the stools are not in a semiliquid condition they must be made so by thoroughly mixing them with distilled water; 5 grams of stool are usually sufficient. The material is then extracted by shaking with an equal volume of ether. The mixture is allowed to stand for fifteen minutes or longer and the supernatant fluid poured off. The remaining fecal material is mixed with one-third its volume of glacial acetic acid and 10 c.c. of ether. The mixture is again thoroughly shaken and set aside for the ethereal layer to separate out, and this then poured off.

The aloin solution which is now used is prepared by dissolving as much aloin as will go on the end of a spatula in one-third of a test-tube of 70 per cent. alcohol; 2 to 3 c.c. of the clear yellow solution are mixed in a test-tube with about the same amount of the acetic ethereal extract and treated with 2 or 3 c.c. of ozonized turpentine (prepared by allowing chemically pure turpentine, such as that of Merck, to stand exposed to the air for at least three weeks), or an equal amount of active hydrogen peroxide. The mixture is thoroughly shaken. If blood is present the reaction may appear in one of several ways: either the whole mixture turns pink, which gradually deepens to a cherry red, or the solution of aloin sinks to the bottom and forms a layer beneath the mixture of ether and turpentine, and this lower layer of aloin in positive tests gradually becomes a deep cherry red. Sometimes if the ether and turpentine are first mixed and the aloin is then allowed to flow gently down the side of the tube, the two sets of fluid will remain separate and a deep-red ring will form at their junction. Not more than fifteen minutes should be allowed for the red color to show itself, for after this the aloin will gradually turn red even if blood is not present. It is necessary to make the aloin solution freshly, for when it stands exposed to the light it changes to about the color that it attains in the reaction when blood is present.

If the test is negative the color remains a light yellow, which becomes red after standing for some length of time.

Guaiac Test.—This test may also be employed, but is not quite so satisfactory as the one preceding. The ethereal extract of the fecal material is prepared as described. The reagent is made by shaking a gram or so of gum guaiac in a test-tube half-full of ether and allowing the mixture to stand until it becomes clear by settling. A couple of c.c. of this solution are added to the same amount of the ethereal extract of the feces and at least an equal volume of hydrogen dioxide is added. The whole is shaken; the hydrogen dioxide settles to the bottom and the ethereal extract floats on top. The blue color (owing to the oxidation of the guaiaconic acid to guaiac blue) of a positive reaction shows itself very quickly in the supernatant fluid, which in a decided reaction becomes a deep blue, that may be somewhat masked by the brown color of the urobilin in the ethereal extract. In such a

case the blue color often becomes a purplish brown, but even this reaction is unmistakable. If the reaction is negative no color change occurs. The guaiac solution must be fresh, but need not be made up daily.

The Benzidin Test.—A small amount of the material to be examined is shaken up in a little water and 3 c.c., approximately, of the unfiltered suspension treated with 2 c.c. of an alcoholic solution of benzidin,[1] 2 c.c. of a 3 per cent. solution of hydrogen peroxide, and a few drops of acetic acid. In the presence of blood an intense green color develops. The test is very delicate, reacting in the presence of blood diluted to 1 to 100,000.

Macroscopic Constituents.—Alimentary Detritus.—Upon gross examination of the feces it is possible to find stones of cherries, grape seeds, woody vegetable fiber, the skins of berries, large pieces of connective tissue, undigested pieces of apple, pear, potato, grains of corn, etc.

The presence of notable amounts of digestible food, such as pieces of muscle tissue, flakes of casein, fragments of amylaceous food, constituting what was formerly spoken of as *lientery*, is always indicative of disturbed gastric or intestinal digestion. It is hence observed in chronic intestinal catarrh, febrile dyspepsia, etc. Occasionally also unaltered food in large amounts is found in the feces, owing to a direct communication between the stomach and the colon, as in cases of perforating ulcer or carcinoma of the stomach.

When fat is present in abnormally large amounts it can usually be recognized with the naked eye. To this condition the term *steatorrhea* has been applied. In typical cases the fat is seen in the form of whitish or grayish masses, varying in size from that of a pea to that of a walnut, which are more or less intimately mixed with the fecal material, and may at first sight be mistaken for flakes of casein. From these it may be distinguished by its chemical reactions and its peculiarly glistening appearance. In other cases stools may be seen in which the fecal column is covered, to a greater or less extent, with a grayish, dense, asbestos-like substance, while the core itself presents the usual color. Nothnagel states that this appearance is referable to congealment of the fat when it is exposed to a lower temperature than that of the body. I have repeatedly observed this appearance in stools which had just been voided and were still warm. In other cases the fat is intimately mixed with the feces, which are colored a light gray throughout. The passage of liquid oil in the absence of fecal material has also been recorded, but it seems doubtful that the oil in such cases entered the body by the mouth. Following the use of oil enemas such stools are, of course, seen.

The elimination of abnormally large quantities of fat may be due to the ingestion of correspondingly large amounts. More frequently,

[1] The solution in question is one saturated by the aid of heat and filtered on cooling.

however, it is referable to pathological conditions. A steatorrhea will thus naturally occur when an insufficient supply of bile is poured into the small intestine, and hence is observed constantly in cases of biliary obstruction. True steatorrhea is also met with in diseases affecting the resorptive power of the small intestine, such as extensive atrophy or amyloid degeneration of the intestinal mucosa, tuberculous ulceration, etc., or in diseases involving the integrity of the lymphatic glands and vessels of the mesentery, as in chronic tuberculous peritonitis, caseous degeneration of the mesenteric glands, etc. In simple catarrhal conditions, however, steatorrhea may also occur, and not only in infants, but, according to my experience, also in adults. The question whether or not steatorrhea is constantly observed in cases of pancreatic disease, as some observers have claimed, may now be answered in the negative, although it must be admitted that the two conditions are very frequently associated. Le Nobel, who has investigated this subject, arrived at the conclusion that the steatorrhea in itself is of little practical importance, but that its association with the absence of products of putrefaction from the stools, the absence of the salts of the fatty acids, and the presence of maltose in the urine, may possibly be regarded as indicating the existence of pancreatic disease.

Garrod and Hurtley have recently described an instance of congenital *family steatorrhea*, two boys, the children of first cousins, having been subject from infancy to true steatorrhea in the absence of other signs denoting pancreatic disease.

Mucus and Mucous Cylinders.—So long as mucus occurs in small particles only, adherent to otherwise normal feces, it is of no pathological significance. Larger amounts are almost always indicative of a catarrhal condition of the colon or rectum, no matter whether the stool is otherwise normal or whether diarrhea exists at the time. Peculiar formations are occasionally seen, viz., so-called *mucous cylinders*, which are passed in large or small fragments in a condition which has been described by Nothnagel as *enteritis membranosa* or *colica mucosa*. Such masses, which at times measure a foot or more in length, are ribbon- or net-shaped, and are frequently passed in the absence of fecal matter, with severe tenesmus. They resemble Curschmann's spirals, but lack the central thread and the Charcot-Leyden crystals. They are probably indicative of chronic constipation associated with catarrh of the colon. Not to be confounded with this condition is the passage of masses of mucus, which do not present the cylindrical form, but which also may be passed with a great deal of tenesmus and in the absence of fecal matter. In cholera Asiatica particles of mucus are seen which resemble grains of rice; their presence was formerly regarded as characteristic of this disease, but they are now known to occur in ordinary catarrhal conditions also.

Biliary and Intestinal Concretions.—Most important from a diagnostic standpoint is the examination of the feces for the presence of biliary concretions, which should never be neglected in cases of colicky, abdominal pain of doubtful origin, whether associated with jaundice or not

When searching for gallstones the feces should be stirred with water and passed through a fine sieve. Biliary concretions may then be found as small, crumbling masses, or as hard stones presenting an irregular contour or the smooth, characteristic facets. In size they may vary from that of a millet seed to that of a pigeon's egg; large stones are rarely passed by the bowel unless perforation has occurred into the intestines and usually into the colon.

Some calculi consist almost entirely of cholesterin, while others are composed essentially of inspissated bile, and still others of calcareous salts. The former are the most common, and are readily recognized by their softness and color, which may be white, grayish, bluish, or greenish. Their specific gravity is lower than that of water. Very frequently they contain a nucleus, composed of earthy sulphates or phosphates.

FIG. 73.—Gallstones: *a*, cholesterin; *b*, pigment stones.

Calculi which consist largely of biliary pigments are brown in color. They are hard, and heavier than water. Frequently they contain traces of copper and zinc (Fig. 73).

Calculi composed of calcareous salts generally present an irregular, roughened contour.

Welch has drawn attention to the not infrequent presence of pure colonies of the Bacillus coli communis in gallstones, apparently forming their nucleus. Typhoid bacilli also have since been observed in their interior, and it appears likely that the formation of gallstones is primarily referable to an invasion of the gall-bladder by such microörganisms. A remarkable case has been reported by Pearce, in which a leptothrix was the only microörganism found in biliary concretions, while in the bile this was present together with the colon bacillus.

Intestinal concretions (enteroliths) are rare and usually come from the appendix. At times they contain some foreign body, such as a grape seed, as a nucleus, upon which calcium and magnesium salts have become deposited.

Fecal calculi or *coproliths* are likewise only rarely seen. They represent inspissated fecal material which has become impregnated with lime and magnesium salts. More commonly they are found at the postmortem table in the cecum, in the haustra of the colon, and in the rectum.

Intestinal sand is also rare. I have seen only 7 cases in the past twelve years. Of its origin nothing is known. The condition is commonly associated with enteritis membranacea. The material presents a brownish color, but may be light green. In 1 case reported by Deetz it was possible to demonstrate the presence of calcium phosphate with traces of calcium oxalate. In another case recorded by Thomson and Ferguson analysis showed 11.7 per cent. of $CaCO_3$; 87.3 per cent. of $Ca_3(PO_4)_2$; insoluble residue (silica), 1 per cent. There was present also a pigment which the writers regard as intermediary between ordinary bile pigment and stercobilin.

Foreign Bodies.—In children, the insane, in cases of hysterial and even in people who are apparently possessed of their normal senses, the physician must be prepared to find at times all kinds of foreign bodies, such as pins, coins, buttons, false teeth, tooth plates with ragged edges, and even dirk-knives, all of which have been known to pass through the alimentary canal. It must not be forgotton, however, that in cases of hysteria bodies may be shown by patients which they claim have passed by the rectum, but which have been wilfully added to the stools, such as snakes, frogs, etc.

MICROSCOPIC EXAMINATION OF THE FECES

General Technique.—The general technique in the microscopic examination of the feces is very simple. Stools that are firm when passed should be stirred up with water to a moderately thin mush. Drops of this material are mounted on a series of slides, covered with cover-glasses, and examined at first with a low power ($\frac{2}{3}$ B. & L.) and then with a medium power ($\frac{1}{6}$ or $\frac{1}{7}$). The survey with the low power furnishes a general idea of the amount of food remnants (muscle fibers, fragments of vegetable material, fat), of the presence of crystals, pus, blood, and eggs of parasites. The higher power ($\frac{1}{6}$ or $\frac{1}{7}$) is reserved for general purposes of verification, to make out details of structure, and the search for the smaller animal parasites (trichomonads, amœba coli, etc.).

If the stools are already thin when passed, no further dilution is necessary. Bits of mucopus or of material showing the presence of blood are generally advantageous for the search for amebas. Musgrave and Clegg, however, recommend that in doubtful cases it is well to administer a saline cathartic and to examine the fluid portion of the resulting movements. In the examination for amebas it is essential that the stools be passed into a warmed bedpan and

examined at once on warmed slides or by the aid of a warmed stage. A convenient form of warm stage, which may be obtained from instrument makers at low cost, is composed of brass and made to be held in position on the stage of the microscope by spring clips. It is about 8 cm. long and 3 cm. broad, and has cemented to a recessed bottom an ordinary glass slip; an opening measuring 1.35 cm. in diameter is in the centre of the stage. To one of the long slides of the brass stage is fitted a projecting stem, about 10 cm. long, to which the heat of a spirit lamp is applied.

If a *search for the eggs of parasites* is to be made it is usually only necessary to mount drops of the stool (diluted to the proper consistence) on slides, and to examine these, covered or uncovered, with the low power of the microscope (B. & L. $\frac{2}{3}$; Leitz 3). If the eggs are scanty it is advisable to concentrate the material by centrifugation, after previous elimination of some of the extraneous fecal material. To this end different methods are available. Bass recommends that the fecal specimen be first washed with water by centrifugation, then two or three times with a solution of calcium chloride having a specific gravity of 1.050. As the eggs are specifically heavier they will be found in the sediment. If for any reason it is desired to separate them still further from other fecal constituents which are heavier than the eggs the sediment obtained as just described is suspended in a solution of calcium chloride having a specific gravity of 1.250 and again centrifugalized. This time the eggs will be found in the surface layer, which may either be examined directly, or it may in turn be diluted with water and the eggs thrown down with the centrifuge. Most remarkable pictures may then be seen if the eggs are at all numerous.

Good results may also be secured by treating a small amount of fecal material with a mixture of equal parts of ether and antiformin. After shaking vigorously for a few minutes the material is passed through a hair filter and centrifugalized, when the eggs will be found in the sediment, relatively free from unimportant fecal constituents.

To preserve the eggs of parasites it has been recommended to proceed as follows: The material is diluted with a large amount of water and allowed to sediment. The supernatant fluid is replaced with a mixture of 95 c.c. of 70 per cent. alcohol and 5 c.c. of glycerin which has been previously heated to the boiling point in a waterbath. After thorough stirring the material is again allowed to settle, the supernatant fluid replaced with fresh fluid and the alcohol evaporated, when the eggs will finally lie in glycerin.

Specimens containing eggs of parasites are readily preserved by the addition of 5 per cent. carbolic acid or of thymol.

Constituents Derived from Food.—Microscopically, indigestible and undigested constituents of food may be seen (Fig. 74), such as

the framework of vegetable material, sometimes still containing starch granules or remnants of chlorophyll; muscle fibers, usually colored yellow and more or less altered in structure. Elastic-tissue fibers are readily recognized by their double contour and bold outlines. Connective-tissue fibers of the white variety can also generally be distinguished; when present in large quantities they are usually indicative of some digestive derangement, unless they are observed following the ingestion of a meal particularly rich in meat. Flakes of casein also are seen frequently.

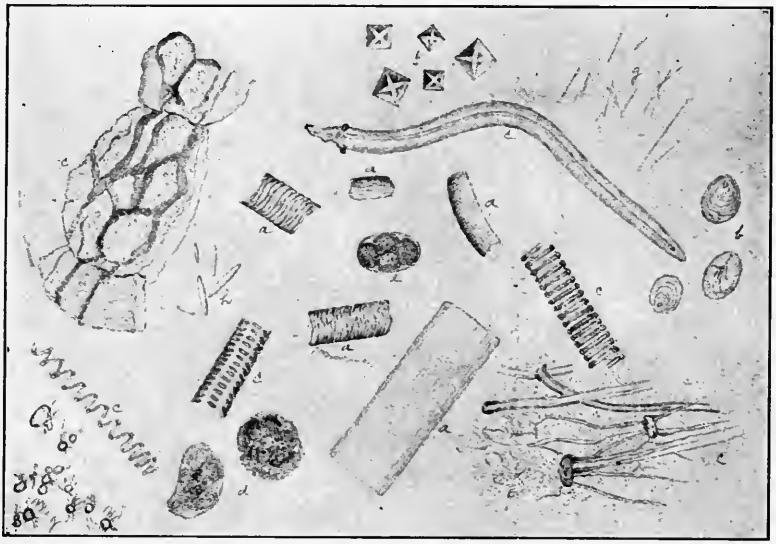


FIG. 74.—Collective view of the feces: *a*, muscle fibers; *b*, starch granules; *c*, vegetable material; *d*, potato cells; *e*, egg of Uncinaria duodenalis; *f*, calcium oxalate crystals; *g*, fatty acid crystals; *h*, Charcot-Leyden crystals.

Muscle fibers are found in every stool whenever meat has been eaten. Under normal conditions, however, they are not numerous, unless particularly large quantities have been ingested. Their appearance under the microscope may vary considerably. On the one hand, fibers are met with which still retain their characteristic features; others are split up either partially or entirely into the well-known disks; but more common than both are more or less roundish, yellow, apparently homogeneous fragments, which at first sight do not resemble muscle fibers in the least. Upon closer investigation, however, their true nature will become apparent. It will then be seen that two of the sides in some portions at least are more or less parallel, and if the specimen is examined with a high-power lens some traces of cross-striation can probably always be discovered.

Isolated starch granules are scarcely ever found under normal

conditions, excepting in young children who have been fed with much starchy material. Starch granules inclosed in vegetable cells are likewise not found, as a general rule, but are more common than the isolated granules. Their presence is easily recognized by treating microscopic preparations with a solution of iodopotassic iodide (Lugol's solution), when the granules or fragments will assume a blue color.

The presence of fat in the feces is quite constant, even in health. It may occur in the form of needle-like crystals, as fat droplets, or as polygonal masses which are highly refractive and often colored yellow or a yellowish red. Their true nature is easily recognized by adding a drop of concentrated sulphuric acid and heating, when they are transformed into the characteristic fat droplets.

The so-called *acholic stools* are usually very rich in fat, and particularly so in cases of biliary obstruction associated with jaundice. At other times the lack of color, as has been mentioned above, is not referable to the absence of hydrobilirubin, but to the presence of colorless decomposition products of bilirubin, such as the leukourobilin of Nencki. In these cases abnormally large quantities of fat are not always present. The conclusion that a stool contains excessive amounts of fat because it is apparently acholic is hence not justifiable unless a microscopic examination has been made.

In pathological conditions it is necessary to determine whether or not food remnants are present in abnormal amount, presupposing, of course, that excessive quantities have not been ingested. It is often possible to draw definite conclusions as to the state of intestinal digestion from the excess of one form of non-digested material over another. The presence of large quantities of undigested starch indicates a catarrhal condition of the small intestine, and it may, indeed, be said that the occurrence of more than traces of this material should be regarded with suspicion. An increase in the number of muscle fibers will, as a rule, likewise be observed under such conditions.

Schmidt and Strassburger have described a special form of intestinal fermentative dyspepsia, in which there is an isolated amylolytic insufficiency, which may be of functional or of organic origin. (See Schmidt's fermentation test below.)

In this connection it is noteworthy that in man extensive disease of the pancreas may exist without seriously disturbing amylolytic digestion.

Schmidt's Fermentation Test.—To obtain a more exact insight into the degree of amylolytic insufficiency of the intestinal tract than is possible from the microscopic study of the feces, Schmidt has proposed a special method which is based upon the continued digestion of the carbohydrates in the feces. The examination is made after the patient has been placed on the following test diet (Schmidt

and Strasburger's test diet No. II): Milk, 1.5 liters; 3½ eggs; strained oatmeal gruel (from 80 grams of oatmeal); 100 grams of zwieback; 20 grams of butter; 20 grams of sugar; 125 grams of steak (raw weight), and 190 grams of potato (raw weight). The distribution of these various articles of food can be arranged as one chooses, or as follows: At 7.30 A.M., ⅜ liter of milk and 2 zwiebacks (each 33 grams); at 10.30 A.M., ⅜ liter of bouillon with ½ egg; at 12 M., ⅜ liter of milk with 1 egg; between 1 and 2 P.M., ½ liter of oatmeal gruel (prepared from 40 grams of oatmeal, 166 grams of milk, 10 grams of sugar, and ½ egg); 100 grams of well-done Hamburg steak (125 grams of raw beef, raw weight) and 12 grams of butter; 250 grams of mashed potato (from 190 grams of potato, 60 grams of milk, and 8 grams of butter); at 4.30 P.M., ⅜ liter of milk, 1 egg, 1 zwieback; at 7.30 P.M., ½ liter of oatmeal gruel as at dinnertime. Before commencing with the test diet, however, it is necessary to demarcate the fecal material by giving a wafer or capsule containing 0.3 gram of powdered carmine. The examination proper is made as soon as the feces are no longer colored red, viz., after from two to three days of the test diet. The necessary apparatus is pictured in the accompanying figure (Fig. 75), which represents one-third of the actual size. For each experiment 5 grams of fresh fecal material are used (the feces being of medium consistence; otherwise a little more or less is taken, corresponding to about 1 gram of dry residue). The material is well stirred with water in the bottle *a*, which is filled entirely and closed with the rubber stopper, care being taken to exclude bubbles of air. Tube *b* is filled with water from the tap and also closed without admission of air. Tube *c* should contain no water; it has a pinhole aperture at the top. The communicating tube *d* is adjusted as shown in the figure. The apparatus is then placed in the incubator at 37° C. for twenty-four hours, not longer. During this time the carbohydrate fermentation will have been completed (Schmidt's Frühgährung). During the evolution of gas, water will be displaced from *b* into *c;* the resulting column is measured and represents the degree of fermentation. The result is regarded as positive if more than a quarter tubeful of gas is obtained. With the test diet in question this would mean a condition approximating the normal. In such an event the patient is placed for two days further on test diet No. I, which differs from No. II only in the absence

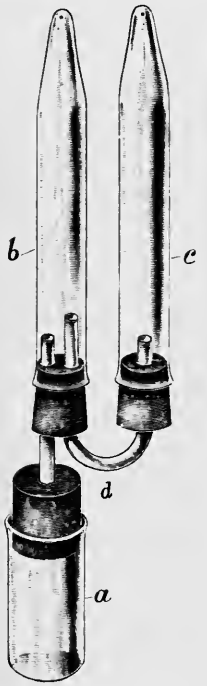


FIG. 75.—Schmidt's fermentation tubes.

of the meat and potato. If then there is still a positive result, the diagnosis of "fermentative dyspepsia" is justifiable. In order to eliminate errors arising from possible formation of gas as the result of albuminous putrefaction the fermenting fecal material should be tested from time to time in a control specimen. If the formation of gas is due to carbohydrate fermentation, there will be an increasing degree of acidity (tested with litmus paper); this increase, however, is not always marked; at any rate, there must be no increasing alkalinity.

Leiner's Test for Casein.—Casein is most conveniently demonstrated with Leiner's method. To this end a small amount of fecal matter is spread on a slide and dried in the air. It is then fixed by heat—passing the specimen through the flame of a Bunsen burner three or four times is sufficient—and stained with a mixture of equal parts of a 0.75 per cent. solution of acid fuchsin and methyl green in 50 per cent. alcohol, the mixture being diluted ten times with water. After fifteen minutes the preparations are placed in distilled water and allowed to remain for one hour or longer. Casein and paracasein are thus stained a pale blue or violet, while similar bodies are practically all colored a light green, or more rarely a yellowish green.

Morphological Elements Derived from the Alimentary Canal.—Epithelium.—Well-preserved cylindrical or goblet cells are only exceptionally found in the feces, while transition forms from the normal cells to mere spindles, in which a nucleus can no longer be recognized, are observed quite constantly. These degenerative changes, according to Nothnagel, are the result of an abstraction of water from the cells, which may alter their appearance to an extent that only the experienced eye is capable of recognizing their true character. Pavement epithelial cells, when present, are derived from the lower bowel.

Epithelial cells when present in large numbers always indicate an inflammatory condition of some portion of the intestinal tract.

Cylindrical epithelial cells are found in abundance in all inflammatory conditions affecting the intestinal mucosa. They are almost exclusively seen embedded in mucus, and it is interesting to note that the cloudy appearance of the mucus is referable to the presence of these elements and not to leukocytes, as is the case in the sputum. When bile-stained specimens are met with, the conclusion is justifiable that the small intestine is involved.

Leukocytes.—Leukocytes are almost always absent in normal stools or present only in very small numbers. Large numbers usually indicate a severe catarrhal, if not an ulcerative, condition of the intestines. Pure pus in large amounts is observed especially in dysentery and in cases in which abscesses have perforated into the gut from adjacent organs or cavities.

Red Blood Corpuscles.—Unaltered red blood corpuscles, according to Nothnagel, are but rarely observed in the feces, no matter how intensely red they may be colored, providing that an ulcerative process affecting the colon or the rectum can be excluded; in that case, as in the severer forms of dysentery, large numbers may be observed. If the hemorrhage has occurred higher up in the intestine, large and small masses of a brownish-red color are seen, which consist of hematoidin. They are mostly amorphous, but in some specimens the characteristic rhombic crystals may be observed. In general, it may be said that the higher the seat of the hemorrhage the darker will be the color of the pigment, and the less the chances of finding well-defined red corpuscles. In such cases recourse must be had to the tests for occult blood (which see).

Crystals.—Needle-like crystals of free fatty acids, and the calcium and magnesium salts of the higher members of this group, occurring either singly or arranged in sheaves, may be found in every stool (Fig. 76). They are of no significance unless present in large numbers. Nothnagel speaks of the frequent occurrence of certain calcium salts (of fatty acids, as he believes) in normal as well as pathological stools. He states that they are almost always bile-stained, and occur in irregular, sometimes elliptical, oval, or circular masses, in which a crystalline structure cannot be distinguished. They are apparently of no importance. Quite common, also, are crystals of neutral calcium phosphate and ammoniomagnesium phosphate, the former occurring in the form of more or less well-defined, wedge-shaped crystals collected into rosettes, the latter presenting the well-known coffin-shape when the stool is mushy, while in firm stools irregular fragments mostly are found. At one time the ammoniomagnesium phosphate crystals were supposed to be characteristic of typhoid stools, but it is now known that they occur in normal feces, as well as under the most varied pathological conditions. Their presence is hence of no diagnostic significance. It is important to note that the neutral phosphates are never stained by bile pigment, and the triple phosphates only in rare instances. Both are easily soluble in acetic acid. Crystals of calcium oxalate may be found in abundance following the ingestion of certain vegetables, such as sorrel and spinach. They are usually found embedded in the vegetable debris. They are readily recognized by their characteristic envelope form, their insolubility in acetic acid, and their solubility in hydrochloric acid Not infrequently they are bile-stained.

Calcium lactate is frequently seen in the stools of children receiving a milk diet; it occurs in the form of sheaves composed of radiating needles. Calcium carbonate is rarely observed, but occasionally occurs in the form of amorphous granules or dumb-bell-shaped crystals. Calcium sulphate crystals are likewise rare, but may be produced artificially by the addition of sulphuric acid, when beautiful

needles and platelets may be observed. Cholesterin, while always present in solution, is rarely observed in crystalline form (Fig. 77). I have found it as such in considerable amount in the stool from a strongyloides case, after this had been kept for several weeks. Hematoidin crystals are never found in normal stools. Charcot-Leyden crystals, according to my experience, are not found in normal stools. They have been described in cases of typhoid fever, dysentery, and phthisis, but are rare in these diseases. In uncinariasis they are more frequently seen, but not in every case. Often they only form after the stool has been kept for some time. They are more likely to be encountered when there are many eggs present than in milder cases. They have further been seen in association with Ascaris lumbricoides, Oxyuris vermicularis, Tænia solium and saginata. In cases of trichocephalus they are but rarely seen, while they are always

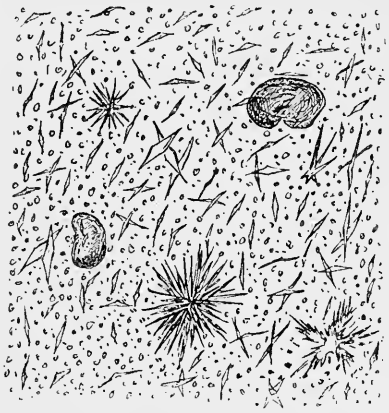


Fig. 76.—Fatty acid crystals obtained from the feces.

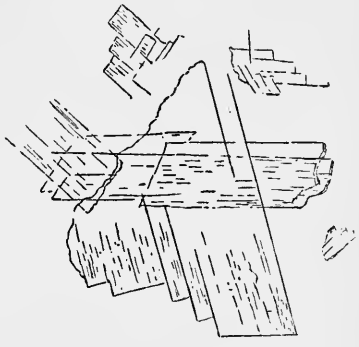


Fig. 77.—Cholesterin crystals.

absent in the case of Tænia nana. According to Leichtenstern their persistence in the feces after the evacuation of what would appear to be a complete tenia should be regarded as indicating the non-removal of the head. I have found them quite numerous in two cases of strongyloides infection. In amebic colitis the crystals have also been observed by Lewis, Lafleur, Amberg, myself and others.

Mucus.—Small hyaline particles of mucus, visible only with the microscope, are not infrequently met with under pathological conditions, and are of diagnostic significance. When bile-stained, their presence is always indicative of disease of the small intestine proper, while colorless particles point to a catarrhal condition of the upper portion of the large intestine or the lower portion of the small intestine. Beginners should be careful not to mistake apparently hyaline particles of vegetable residue for mucus. Mucus never yields a

blue color when treated with iodine, or iodine and sulphuric acid, and examination with a higher power will show the entire absence of any definite structure. Both forms, viz., colorless and colored particles, are found intimately mixed with the feces, and may be very abundant. In addition to these forms Nothnagel has described the occasional occurrence of large numbers of roundish or irregular, very pale hyaline or opaque formations, which are devoid of all structure. Some specimens are homogeneous, while others present a distinct rimous appearance. They have been found only in liquid stools, and are apparently of no diagnostic significance. To judge from their optic behavior, they probably consist of mucus.

BACTERIOLOGY OF THE FECES

Bacteria constitute the greater portion of the fecal solids. Their number is truly enormous. Sucksdorff found in his own person that on an average 53,124,000,000 were eliminated in the twenty-four hours under normal conditions. If we recall the strongly bactericidal power of the gastric juice, such an observation must at first sight appear surprising. It should be remembered, however, that large amounts of the ingesta are carried into the small intestine at a time when hydrochloric acid has not yet appeared in the free state.

On the whole, the bacteriological flora of the intestinal contents is fairly constant, but, as in the other cavities and channels of the body where bacteria are invariably met with, transient guests are also not uncommon. The majority of the bacteria which are here encountered are, as a general rule, harmless; but it is important to note that under suitable conditions a number of these may develop pathogenic properties. Broadly speaking, the bacteria which may be found in the feces can be divided into two classes, viz., into alkali producers and acid producers. Many of these forms have been described for the first time by Ford, and the following schema, which gives a very good idea of the numerous individual types, although not complete, is taken from his excellent work:

Alkali Producers

Group I. Organisms producing alkali in litmus milk; not liquefying any media; not fermenting carbohydrates to the point of acidity. *Fœcalis alkaligenes*, or *Petruschky group*. Represented by:

Bacillus alkaligenes.

GROUP II. Organisms producing alkali; not liquefying any media; fermenting carbohydrates to the point of acidity, but no gas. *Dysentericus,* or *Shiga group.* Represented by:

Bacillus dysenteriæ.
Bacillus pseudodysentericus, Müller.
Bacillus typhi.
Bacillus acidophilus.

GROUP III. Organisms producing alkali; not liquefying any media; fermenting the carbohydrates with the production of acidity and gas. *Hog cholera,* or *suipestifer group.* Represented by:

Bacillus alkalescens, Ford; ferments dextrose, saccharose, and lactose.
Bacillus subalkalescens, Ford; ferments dextrose, saccharose, and lactose.
Bacillus enteritidis, Gärtner; ferments dextrose.
Bacillus galactophilus, Ford; ferments saccharose and lactose.

GROUP IV. Organisms producing alkali; liquefying gelatin; ferment ing carbohydrates with the production of acid and gas. *Entericus group.* Represented by:

Bacillus entericus, Ford; ferments dextrose, saccharose, and lactose.
Bacillus subentericus, Ford; ferments dextrose and lactose.

GROUP V. Organisms producing alkali; liquefying gelatin, casein, and blood serum; fermenting carbohydrates with the production of acid and gas. *Proteus,* or *Hauser group.* Represented by:

Bacillus plebeius, Ford; ferments dextrose, saccharose, and lactose.
Bacillus infrequens, Ford; ferments dextrose and lactose.
Bacillus vulgaris, Hauser; ferments dextrose and saccharose.

GROUP VI. Organisms producing alkali; liquefying various media, but not fermenting carbohydrates to the point of acidity. *Booker group.* Represented by:

Bacillus recti, Ford; liquefies gelatin.
Bacillus pylori, Ford; liquefies gelatin and casein.
Bacillus ceci, Ford; liquefies gelatin, casein, and blood serum.
Bacillus bookeri, Ford; liquefies gelatin, casein, and blood serum.
Bacillus pyocyaneus.

Acid Producers

GROUP I. Organisms acidifying and coagulating milk; not liquefying any media; not fermenting carbohydrates to the point of acidity. *Fæcalis oxygenes,* or *Bienstock group.* Represented by:

Bacterium oxygenes, Ford.
Bacterium Bienstock, Schröter.

Group II. Organisms acidifying and coagulating milk; not liquefying any media; fermenting carbohydrates to the point of acidity, but no gas. *Acidoformans*, or *Sternberg group*. Represented by:

Bacillus oxyphilus, Ford.

Bacterium acidoformans, Sternberg.

Bacterium minutissimum, Migula.

Group III. Organisms acidifying and coagulating milk; not liquefying any media; fermenting carbohydrates with the production of acidity and gas. *Coli*, or *Escherich group*. Represented by:

Bacillus coli, Migula; ferments dextrose and lactose.

Bacillus communior, Ford; ferments dextrose, saccharose, and lactose.

Bacterium aërogenes, Migula; ferments dextrose, saccharose, and lactose.

Bacterium duodenale, Ford; ferments dextrose and lactose.

Group IV. Organisms acidifying and coagulating milk; liquefying gelatin and fermenting the carbohydrates with the production of acidity and gas. *Liquefaciens*, or *Eisenberg group*. Represented by:

Bacillus gastricus, Ford; ferments dextrose, saccharose, and lactose.

Bacillus subgastricus, Ford; ferments dextrose and lactose.

Bacterium liquefaciens, Eisenberg; ferments dextrose, saccharose, and lactose.

Bacterium subliquefaciens, Ford; ferments dextrose and lactose.

Group V. Organisms acidifying and coagulating milk; liquefying gelatin, casein, and blood serum, and fermenting the carbohydrates with the production of acidity and gas. *Cloacæ*, or *Jordan group*. Represented by:

Bacillus cloacæ, Jordan; ferments dextrose, saccharose, and lactose.

Bacillus subcloacæ, Ford; ferments dextrose and lactose.

Bacillus iliacus, Ford; ferments dextrose and saccharose.

Group VI. Organisms acidifying and coagulating milk; liquefying various media; fermenting the carbohydrates with the production of acidity, but no gas. *Dubius*, or *Kruse group*. Represented by:

Bacillus chylogenes, Ford; liquefies gelatin.

Bacterium chymogenes, Ford; liquefies gelatin.

Bacillus leporis, Migula; liquefies gelatin and blood serum.

Bacillus dubius, Kruse; liquefies gelatin, blood serum, and casein.

Bacillus jejunalis; liquefies gelatin, blood serum, and casein.

All the above are non-pigment, non-spore-bearing organisms. In addition to these the following pigment-producing and spore-bearing organisms have been isolated:

Pseudomonas aëruginosa, Schröter.

Pseudomonas ovalis, Ravenel.

Bacterium Havaniense, Sternberg.
Bacterium lutescens, Migula.
Bacterium anthracoides, Hüppe and Wood.
Bacterium implectans, Burchard.
Bacillus cereus, Frankland.
Bacillus mycoides, Flügge.

The above list indicates the various organisms which have thus far been isolated from the intestinal contents. Many other forms exist, but have not yet been cultivated, as they do not grow on the artificial media which are now in use.

Those which interest us more especially from the pathological side are the dysentery bacillus, the typhoid bacillus, the paratyphoid group, the Bacillus acidophilus, B. (proteus) vulgaris, B. pyocyaneus, B. coli communis, B. lactis aërogenes, V. choleræ, and the tubercle bacillus. These organisms and their cultivation will be considered in detail in Chapter XI.

Fungi.—Fungi, with the exception, perhaps, of the Oidium albicans, which has at times been observed, are rarely found in the feces.

Schizomycetes.—Saccharomyces cerevisiæ is one of the normal constituents of the feces, and is found in its characteristic forms, three or four buds, however, being but ordinarily observed. Owing to the glycogen present in their substance, they assume a mahogany color when treated with a solution of iodopotassic iodide. They should not be confounded with a class of bacteria which closely resemble the saccharomyces in general appearance, but are colored blue when treated in the same manner.

ANIMAL PARASITOLOGY OF THE FECES

Classification.—The animal parasites which may be met with in the feces may be classified as follows:

A Protozoa:
- I. Rhizopoda:
  - Amœbina.
    - Amœba: Entamœba dysenteriæ; E. coli; Paramœba hominis.
- II. Flagellata (Mastigophora):
  - *a.* Polymastigina.
    - 1. Trichomonas: Trichmonas intestinalis.
    - 2 Lamblia: Lamblia intestinalis.
  - *b.* Protomonadina.
    - 1. Cercomonas: C. hominis.
- III. Sporozoa:
  - 1. Gregarinida.
  - 2. Coccidiida.
- IV. Infusoria:
  - 1. Balantidium: B. coli.

B. Platyhelminthes (Flat worms):
- I. Trematodes:
  - 1. Paramphistomidæ: Gastrodiscus hominis.
  - 2. Fasciolidæ:
    - *a.* Fasciola: F. hepatica.
    - *b.* Fasciolopsis: F. buski; Distomum rhatonisi.
    - *c.* Paragonimus: P. westermani.
    - *d.* Opisthorchis: O. fellineus; O. sinensis; O. noverca.
    - *e.* Cotylogonimus: C. heterophyes.
    - *f.* Dicrocœlium: D. lanceolatum.
  - 3. Schistosomidæ:
    - *a.* Schistosomum: S. hæmatobium; S. japonicum.
- II. Cestodes (Tapeworms):
  - 1. Bothriocephaloidea:
    - *a.* Dibothriocephalus: D. latus.
    - *b.* Diplogonoporus: D. grandis.
  - 2. Tæniidæ:
    - *c.* Dipylidium: D. caninum.
    - *d.* Hymenolepis: H. nana; H. diminuta; H. lanceolata.
    - *e.* Davainea: D. madagascariensis.
    - *f.* Tænia: T. solium; T. marginata; T. serrata; T. saginata; T. africana; T. echinococcus.
- III. Nematodes (Threadworms):
  - 1. Anguillulidæ:
    - *a.* Rhabditis.
    - *b.* Anguillulina.
  - 2. Angiostomidæ:
    - *c.* Strongyloides: S. intestinalis (stercoralis).
  - 3. Trichotrachelidæ.
    - *d.* Trichocephalus: T. trichuris.
    - *e.* Trichinella: T. spiralis.
  - 4. Strongylidæ:
    - *f.* Ankylostoma: A. duodenale, Uncinaria americana.
  - 5. Ascaridæ:
    - *g.* Ascaris: A. lumbricoides; A. canis; A. maritima.
    - *h.* Oxyuris: P. vermicularis.

Protozoa.—The *rhizopoda* are essentially characterized by the fact that locomotion does not take place by the aid of independent organs, but by means of pseudopodia, viz., protoplasmic processes which the animal is capable of protruding from any portion of its body. Six orders have been described by zoölogists, but only one, or possibly two, have thus far been found in the feces. Whether or not representatives of the *monera* occur in the feces of man is still an open question. If so, they are apparently of no pathological significance. Of the *amœbina*, on the other hand, a most important member has been found, viz., the Entamœba dysenteriæ.

Entamœba Dysenteriæ, s. Histolytica (Schaudinn): *syn.*, **Amœba Coli** (Lösch).[1]—In 1875 Lösch discovered in the stools of dysenteric patients actively moving cell-like bodies of a roundish, pear-shaped, oval, or irregular form. He did not regard these as the cause of the disease, however, but looked upon them as only accidentally present. Similar bodies were observed in Hong-Kong by Normand in cases of colitis; and also by v. Jaksch. Sansino found them in a case in

[1] Craig has recently proven the identity of E. histolytica with E. tetragena.

Cairo, and Koch in East Indian dysentery. It is interesting to note that Koch was the first to suspect the existence of a definite relation between dysentery and these organisms. Cunningham claims to have found amebas frequently in the stools of cholera patients at Calcutta, and Grassi in normal stools, but especially abundant in cases of chronic diarrhea. Whether all these observations are correct, and whether the organisms observed were identical in all cases, is, of course, difficult to say. So much is certain, that the subject was still in a very unsettled state when Kartulis announced "that dysentery and tropical liver abscess associated with dysentery are caused by the presence of the Amœba coli," basing his conclusion upon an examination of 500 cases. The fact that this parasite was absent in all other intestinal diseases, such as typhoid fever, intestinal tuberculosis, the ordinary forms of diarrhea, etc., spoke strongly in favor of Kartulis' view. His conclusions have since been confirmed by numerous observers the world over, and it may be regarded as an established fact that a certain type of dysentery which is common in tropical, subtropical, and to a certain extent even in temperate climates, is due to infection with amebas. In contradistinction to the bacillary form of dysentery, the amebic variety tends to a certain degree of chronicity, and is further characterized by the frequency with which solitary liver abscess develops as a complication.

The size of the amebas averages 35μ. When at rest their outline is, as a rule, circular, occasionally ovoid; but when in motion they present the extremely irregular contour of moving ameboid bodies (Plate XVIII). The protoplasm can be differentiated into a translucent, homogeneous ectosarc or mobile portion, and a granular endosarc, containing the nucleus, vacuoles, and granules. Within the endosarc the vacuoles constitute the most striking feature.

The organism may reproduce itself by fission or by encystment, the former being the more common. When reproduction by encystment is to take place the protoplasm develops crystalloids and the nucleus divides twice in succession, the resultant body thus containing four nuclei. Such cysts usually measure from 7 to 12μ, though occasional specimens may attain a diameter of 14μ. In this respect the E. dysenteriæ differs markedly from the E. coli, whose propagative cysts are much larger (25 to 30μ) and contain eight nuclei, instead of four. Sometimes the interior seems to be made up of a series of closely set, clear vesicles of pretty uniform size. As a rule, one or two larger vacuoles are present, the edges of which are not infrequently surrounded by fine, dark granules. True contractile vesicles displaying rhythmic pulsations have not been observed, although the vacuoles may at times be seen to undergo changes in size. In some the nucleus is quite distinct, while in others it may be altogether invisible. The protoplasm of the amebas is strongly basophilic.

Most distinctive are the movements of these bodies. From any part of the surface a rounded, hemispherical knob will project, and with a rapid movement the process extends and the granules in the interior flow toward it. In these movements the clear ectosarc seems to play the most important part. The organisms are actively phagocystic and often contain red corpuscles, bacteria, and crystals. Very curiously cysts are rarely seen during the height of the disease, but appear in considerable numbers during convalescence.

Various attempts have been made to cultivate the Amœba coli, but on the whole the results have not been satisfactory. In every attempt in this direction adequate bacterial symbiosis must be secured. The most comprehensive work in this direction has been done by Musgrave and Clegg. The medium which they recommend has the following composition and is prepared as ordinary agar:

Agar	20.0 pro liter.
Sodium chloride	0.3 to 0.5 "
Beef extract	0.3 to 0.5 "

The final product is most universally satisfactory when 1 per cent. alkaline to phenolphthalein, to which end it is recommended to start with an initial alkalinity of 1.5 per cent.

Tubes of this medium are plated and the surface slightly smeared with material selected from feces containing amebas. The first plates must be watched frequently under the microscope, and as soon as it is found that amebas have developed (twenty-four hours to four or five days) transplants must be made, as otherwise they are liable to die

To demonstrate amebas in stools it has been generally suggested to procure bits of mucus or mucopus for examination. Musgrave and Clegg recommend that the patient be given a saline cathartic and that the examination be made from the fluid portion of the stool. Drops of this are mounted, covered with cover-glasses, and examined with a $\frac{1}{6}$. The diagnosis of amebiasis should then only be made if motile amebas are encountered. Resting or encysted forms may be mistaken for epithelial cells, swollen leukocytes, etc.

Not infrequently some of the organisms are found containing one or more red cells (Plate XVIII).

Staining is not at all essential for the purpose of demonstrating amebas in the stool. The examination of the fresh material is much more satisfactory and far less likely to lead to errors of diagnosis.

Very pretty pictures may, however, be obtained by vital staining with neutral red (Plate XVIII). To this end it is only necessary to run a drop of a dilute solution of the dye under the cover-glass, when it will be seen that the young, actively motile amebas take up the stain without losing their motility. They can then be readily watched in their movements.

The preparation of stained permanent specimens is not very sat-

FIG. 78.—Entamebæ tetragena. Note nucleus in both entamebæ. Living specimen. × 750. (From Bull. 1, 1913, Surgeon-General's Office.)

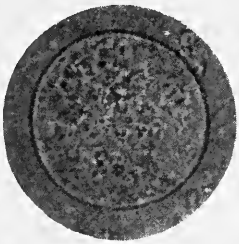


FIG. 79.—Entameba Coli. Encysted form. Two or more of the nuclei may be seen if the photomicrograph is examined carefully. Living specimen. × 750. (From Bull. 1, 1913, Surgeon-General's Office.)

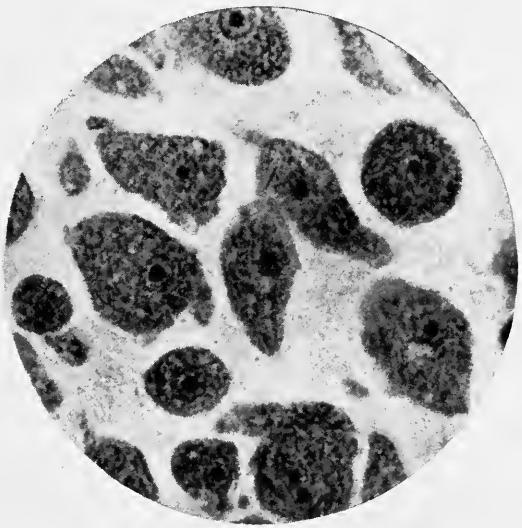


FIG. 80.—Amœba of Limax type. From a culture. Note the character of the nucleus. × 750. (From Bull. 1, 1913, Surgeon-General's Office.)

isfactory. They are made like blood films and colored with one of the modifications of the Romanowsky dye.

When older material only is available it may be difficult to arrive at a satisfactory conclusion. Sometimes it is possible to cause the amebas to move again by warming the stool in an open dish at body temperature, but more often they are dead. Attention should then be especially directed to ameba-like structures containing red blood cells. If such are found the inference that the cell is a dead ameba is usually warrantable.

Entamœba Coli (Schaudinn).—This is not to be confused with the Entamœba dysenteriæ. It is smaller than the Entamœba dysenteriæ, the size varying between 10 and 15 μ. It is opaque, gray in color, and provided with a distinct nucleus. The ectoplasm is usually not visible. The movements are much more sluggish and the tendency to phagocytosis much less marked. Propagation cysts contain eight nuclei instead of four, as in the case of E. dysenteriæ, and are much larger, measuring from 25 to 30μ. It is considered to be non-pathogenic. In the Philippines it is apparently quite common. Craig found 65 per cent. of normal individuals infected with it, using saline purgatives to produce diarrheal discharges, as recommended by Musgrave.

Paramœba Hominis (Craig).—Craig observed organisms which apparently occupy a position intermediary between amebas and flagellates in several cases of severe diarrhea occurring in the Philippine Islands. In one stage of its existence the parameba is capable of active progressive locomotion and is much larger than the trichomonas in the resting stage. In the flagellate stage it is distinguished from the corresponding stage of trichomonas by the absence of an undulating membrane, the presence of a single flagellum, and its circular form. The question of its pathogenicity has not been decided.

The *Flagellata s. mastigophora* differ from the rhizopoda in being provided with from one to eight flagella, which serve as organs of locomotion and possibly also for the apprehension of food particles. Representatives of two orders only, viz., the *monadina* and *isomastigoda*, have been found in the feces. Of the monadina in turn only one family, viz., the cenomonadina, and of the isomastigoda only two families, the tetramitina and polymastigina, are represented.

The *cenomonadina* are small, oval, frequently elongated bodies, provided with one long flagellum at the anterior end, at the base of which food vacuoles are situated. At the posterior end ameboid movements may be observed, and there can be no doubt that the taking up of food, to some extent at least, also occurs by the aid of pseudopodia. To this family belongs the *cercomonas* of Davaine and Lambl. The *tetramitina* are small, elongated bodies, provided with four flagella and a lateral, undulating membrane, which was

formerly mistaken for a posteriorly directed flagellum. The tail end of the organism tapers to a point. The nucleus is located at the base of the flagella. To this family belongs the parasite which was first discovered by Donné in the vagina, and which later was found also in the feces, and which has been variously designated as Trichomonas hominis, Cercomonas coli hominis, etc.

The *polymastigina* are small, somewhat oval bodies, provided with two or three flagella, situated either anteriorly or laterally—two or three on each side—while at the same time two additional flagella issue from the posterior end, which may either be rounded off or taper to a point. To this family belongs the Megastoma entericum of Grassi.

The question whether or not the flagellate bodies are of pathological importance still remains *sub judice*. They are apparently met with only in diseases associated with diarrhea, and it appears that in some cases at least this is directly dependent upon their presence; in others the impression is gained as though they merely maintained an already existing diarrhea referable to other causes; while in a third class of cases no relation can be discovered between their presence and the disease in question. Cohnheim has pointed out that living infusoria in the feces may be a symptom of a primary chronic stomach affection (gastritis, usually the atrophic form). According to the same writer, encysted infusoria may also be found in the feces of healthy individuals, but in such cases we may assume that at some time previously a gastritis or a gastro-enteritis has existed. He thinks they have no pathogenic significance, and are merely of symptomatic-diagnostic interest.

Cercomonas of Davaine-Lambl: *syn.*, Cercomonas hominis (Davaine); monas (Marchand); Monas lens (Grassi); Monas monomitina (Grassi). Tetramitus Mesnili (Wenyon). The organism closely resembles the Trichomonas intestinalis and has frequently been mistaken for it. The points of difference are readily brought out in stained specimens (iron-hematoxylin). The organism is pear-shaped, measuring about 14μ in length by 5 to 6μ in breadth. At the anterior end a large mouth-like depression will be observed—the cystostoma from which three anteriorly directed flagella issue. The adult organism (Fig. 81) is oval or roundish in form, and provided anteriorly with a single long flagellum and posteriorly with a tail-like appendage. Its length varies from 0.005 to 0.014 mm. The younger forms are pear-shaped or S-shaped, and sometimes irregular in outline; the flagellum is then either absent or rudimentary.

Upon prolonged observation it will be seen that the adult parasite loses its flagellum and may protrude a protoplasmic process instead, while vacuolation occurs at the same time, indicating approaching death.

Trichomonas, Donné: *syn.*, Trichomonas vaginalis (Donné); Trichomonas hominis (Grassi); monocercomonas (Grassi); cimænomonas (Grassi); Protorycomyces coprinarius (Cunningham and Lewis); Cercomonas coli hominis (May); Trichomonas intestinalis (Leuckart and Roos); Cercomonas s. Bodo urinarius (Künstler). The parasite

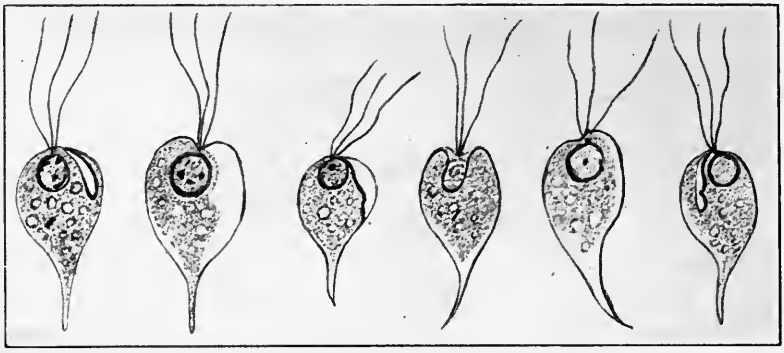


Fig. 81.—Cercomonas intestinalis. (From Brumpt.)

(Fig. 82) is pear-shaped and measures from 10 to 15μ in length by 7 to 10μ in breadth. From its anterior pole flagella are given off, which are almost as long as the organism itself. Three of these are directed forward, while the fourth is turned posteriorly, and clinging closely to the body of the organism, forms an undulating membrane which extends to the posterior pole. This may be rounded off or

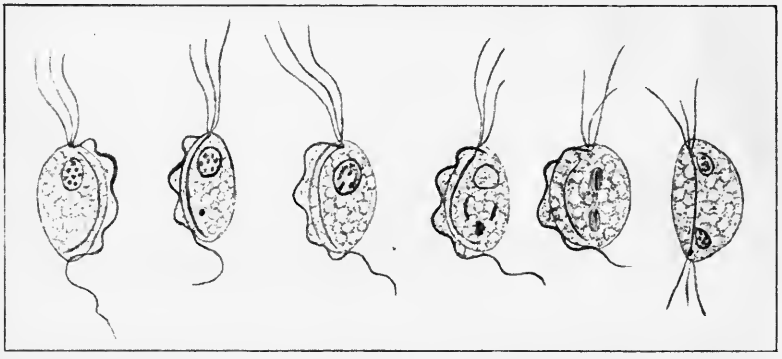


Fig. 82.—Trichomonas intestinalis. (From Brumpt.)

tapers to a tail-like appendage. The membrane is best seen when the movements of the flagella have ceased, as in specimens fixed in mercuric chloride solution (1 to 5000). The nucleus is situated at the base of the flagella, but is usually visible only in stained specimens (methylene blue). At times the organisms may be observed to assume

an ameboid form; the movements of the flagella have then ceased, and pseudopodia-like processes are protruded. The parasite is identical with the trichomonas which has been found in the vagina and in the urine. When present in the feces the organism is usually seen in enormous numbers. Not infrequently it is found associated with other intestinal parasites. Its habitat is the large intestine (cecum) where it reproduces itself by longitudinal division. Regarding its pathogenic significance it would appear that it can only develop in connection with some irritative factor, but that when once established it may keep up a diarrhea indefinitely.

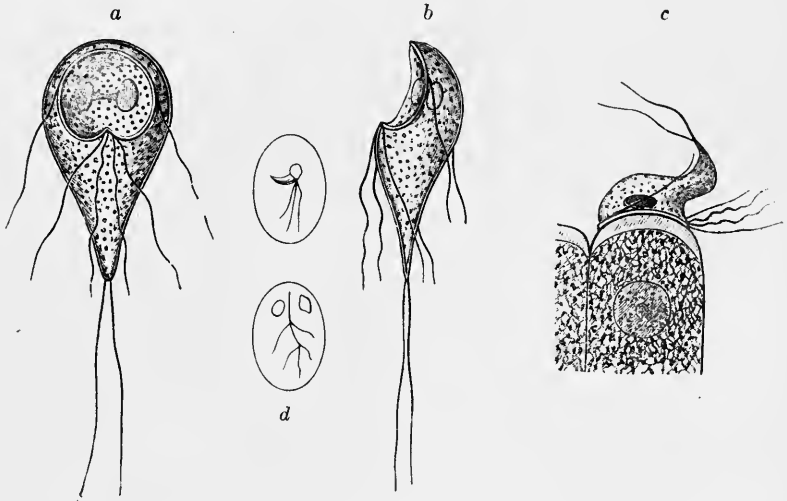


FIG. 83.—Lamblia intestinalis: *a*, front view; *b*, side view; *c*, organism attached to an epithelial cell. (Mosler.) *d*, encysted form (personal observation).

Lamblia intestinalis (Blanchard): *syn.*, Megastoma entericum; Cercomonas intestinalis (Lambl); Megastoma intestinale (Bütschli); Dimorphus muris (Grassi). The parasite (Fig. 83) is pear-shaped, and measures from 0.01 to 0.021 mm. in length by 0.0075 to 0.05 mm. in breadth. In its anterior portion a more or less well-marked depression can be made out, which constitutes the peristome or mouth opening of the organism. It is provided with eight flagella, grouped in pairs. The first pair originates on the sides of the peristome and is directed backward. The second and third pair are situated somewhat posteriorly and are likewise directed backward, while the fourth pair issues from the tapering tail end of the body. In fresh specimens the eighth flagella can usually not be made out, as the third and fourth pair are frequently agglutinated. The best results are obtained when the organism has been killed with mercuric chloride solution. The individual flagella vary from 0.009 to 0.014 mm. in

length. In the anterior portion of the peristome two round, hyaline bodies can be recognized, which represent nuclei. Vacuoles are absent, and nutrition occurs through osmosis, the parasite adhering to epithelial cells by its periostome. When treated with fixing solutions the chitinous envelope can be readily recognized. In the encysted form the organism is oval and measures from 11.5 to 12.5μ.

In the United States the organism seems to be not infrequent in the Carolinas (Stiles). In other States it is apparently rare. Emerson observed one case at the Johns Hopkins Hospital, and during the past year I have found the organism in two cases—children—who had not been beyond the boundaries of Maryland. In both of my cases encysted forms only were found, but these were present in enormous numbers.

Grassi observed the organism in mice, rats, cats, dogs, rabbits, and sheep. The *ciliata*, as the term indicates, carry cilia, and of these only one member, belonging to the *holotricha*, is found in the feces, namely, the Balantidium coli.

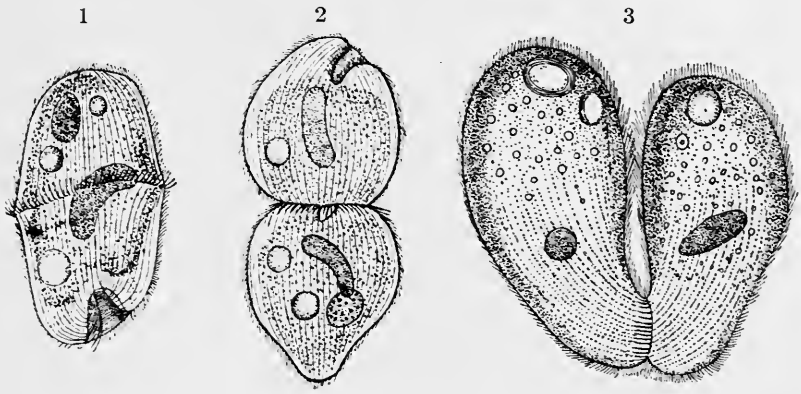


FIG. 84.—Balantidium coli: 1, 2, division; 3, conjugation. (After Leuckart, from Döflein.)

Balantidium coli, Stein: *syn.*, Paramœcium coli (Malmsten). The organism is oval and measures from 70 μ to 110 μ in length by 60 μ to 72 μ in breadth. It is covered entirely with fine, actively motile cilia, which are grouped most densely about the funnel-shaped mouth, while at the anus only a few are seen. An ectosarc and an endosarc may be distinguished, and the parasite possesses the power to change its shape, and may appear quite round. In its interior we find a large, somewhat kidney-shaped nucleus, two contractile vesicles, and frequently fat droplets, starch granules, etc. (Fig. 84).

The parasite is probably pathogenic, but comparatively uncommon outside of Sweden, Finland, and Russia. Infection occurs through the dejecta of swine. Strong and Musgrave report that in their case blood examination showed a relative increase of the eosinophiles.

From 200 to 300 organisms have been encountered in a single drop of the liquid feces.

The fourth class of protozoa, viz., the *gregarina* or *sporozoa*, is also said to be represented in the human feces. The coccidia and psorosperms belong to this order. They are oval bodies, measuring about 22μ in length, and contain in their interior a large number of small nuclei arranged in groups. They are entirely devoid of organs of locomotion, and obtain their nutriment by endosmosis. Reproduction occurs in a common capsule, which bursts at a certain time and sends forth a whole generation of fully developed organisms. In human pathology they have become of interest in so far as certain observers have ascribed to them à role in the etiology of neoplasms. A disease of the liver analogous to the *psorospermiasis* of rabbits has also been described in man, and parasites belonging to the same order have been observed in the skin.

FIG. 85.—Segments of tapeworms: *a*, Tænia saginata; *b*, Bothriocephalus latus; *c*, Tænia solium.

Cestodes.—Tænia saginata, Goeze: *syn.*, T. mediocanellata (Küchenmeister); T. incruris (Huber); T. dentata (Nicola). This parasite (Fig. 85) is the most common tapeworm in Europe and North America. Infection occurs through the ingestion of measly beef. Its length varies from 4 to 8 m. The head, which is devoid of a rostellum, is surrounded by four pigmented suckers, each of which is encircled by a dark line. The individual segments are quite thick and opaque, and diminish in length as the head is approached, the largest

measuring from 2 to 3 cm. They are each provided with a very much branched uterus, which opens laterally, the primary branches numbering about twenty on each side (Fig. 85). The ova are oval in form, of a brown color, and usually enclosed in a vitelline membrane (Plate XIX). They measure 30 to 40μ in length by 20 to 30μ

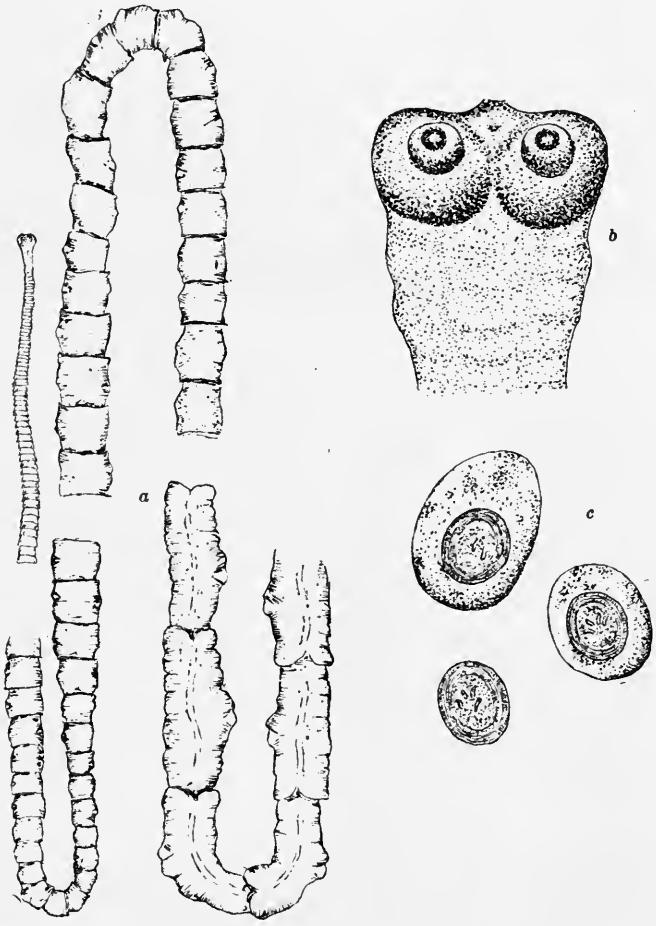
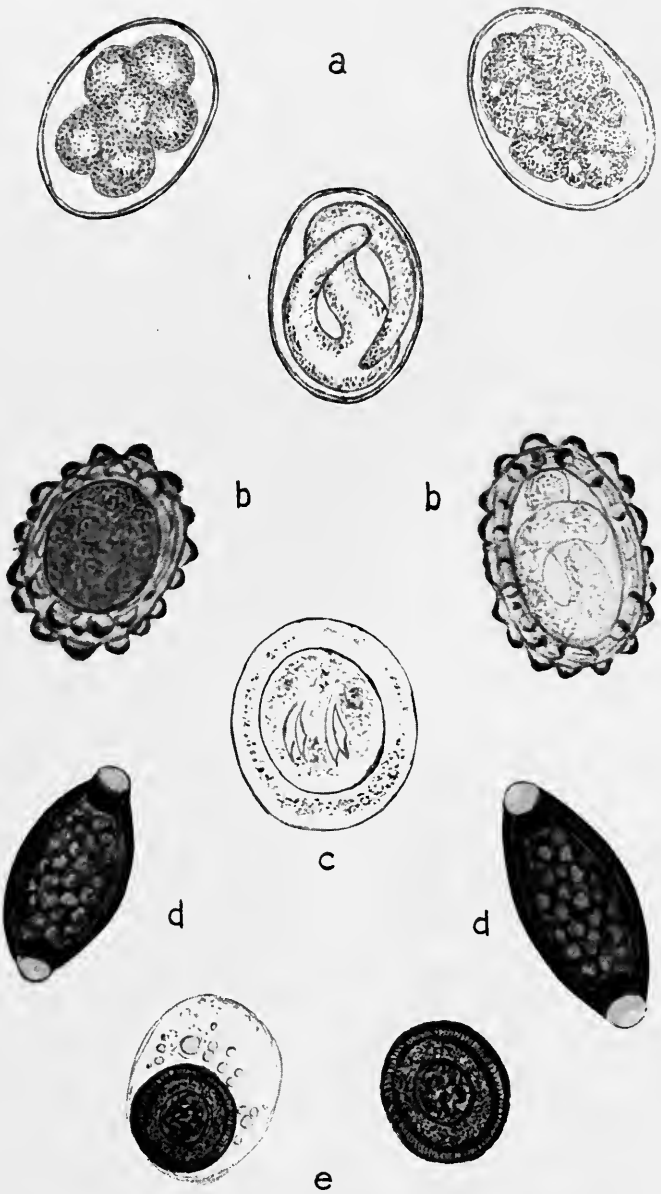


FIG. 86.—Tænia saginata: *a*, natural size; *b*, head, much enlarged; *c*, ova, much enlarged.

in breadth. Upon careful observation a double contour with delicate, radiating striæ can be discerned. In the interior the hooklets of the embryos, which are lost in the adult worm, are seen embedded in a brown, granular material.

The diagnosis is mostly made by the patient when segments are found in the stools. In contradistinction to the segments of Tænia solium those of Tænia saginata are actively motile and readily

PLATE XIX



Eggs of Intestinal Parasites.

a, uncinaria americana; *b*, ascaris lumbricoides; *c*, hymenolepis nana; *d*, trichocephalus trichiurus; *e*, tænia saginata.

migrate from the intestine through the anus to the outer world. At times long fragments of the worm are evacuated. In doubtful cases the eggs should be looked for; they are readily seen with a low power (⅔ Bausch & Lomb).

Very curiously multiple infections are very rare and we must assume that the first worm that succeeds in establishing itself in the small intestine creates an immunity against the development of other worms of the same species.

The larval form of Tænia saginata, the so-called Cysticercus tæniæ saginatæ (Leuckart), or the Cysticercus bovis (Cobbold), has been encountered in cattle, the Rocky Mountain "antelope," the llama, and the giraffe. In the human being it has not been observed.

Tænia solium, Rudolphi: *syn.*, T. cucurbitina, plana, pellucida, Goeze. This parasite (Fig. 87) is far less common in this country than the Tænia saginata, and may, indeed, be regarded as a curiosity. In Germany, also, it is only rarely met with now, while formerly it was the most common tapeworm in that country. This change is undoubtedly owing to the fact that raw pork is now eaten less frequently and that meat inspection is carried out more vigorously. In Asia and Africa it is more common.

FIG. 87.—Head of Tænia solium. × 45. (Leuckart.)

Tænia solium is usually much shorter than Tænia saginata, rarely exceeding 3.5 m. in length. Most characteristic is the head, which is provided with four pigmented suckers and a rostellum, furnished with from twenty-four to twenty-six hooklets arranged in a double row. The mature segments measure from 1 to 1.5 cm. in length by 6 to 7 mm. in breadth, and contain a uterus which has only five to seven branches, thus differing greatly from that of Tænia saginata (Fig. 85). The ova are round, of a brownish color, and surrounded with a thick, radially striated membrane; in their interior the hooklets of the embryos can usually be made out. They measure 31 to 56 μ in diameter and are frequently surrounded by the vitelline membrane. They are readily found in the feces, and should be looked for in doubtful cases. The segments themselves never leave the intestinal canal through active movements on their own part, as may happen in the case of Tænia saginata, but are exclusively evacuated with the feces. As they rarely appear in large groups

(viz., more than 5 or 6) the existence of the parasite may readily be overlooked.

Single infections are the rule, but multiple infections occasionally occur and apparently more frequently so than in the case of Tænia saginata. In one instance 59 adult tænias were counted in the same individual, and in another 22 were evacuated at one time.

The larval form of this tapeworm, the *Cysticercus cellulosæ*, has been found in swine, the wild boar, in monkeys, in the brown bear, in the dog, etc. At times, though rarely, an auto-infection with the proglottides of Tænia solium has also been observed in the human being. Under such conditions the embryos of the worm are set free in the stomach, and may then migrate into various parts of the body, where they become encysted. Most commonly the cysticerci are found in the skin; they have, however, also been observed in the heart, the lymph glands, liver, bones, tongue, spinal canal, the brain, and the eyes. I have had occasion to observe a case of this

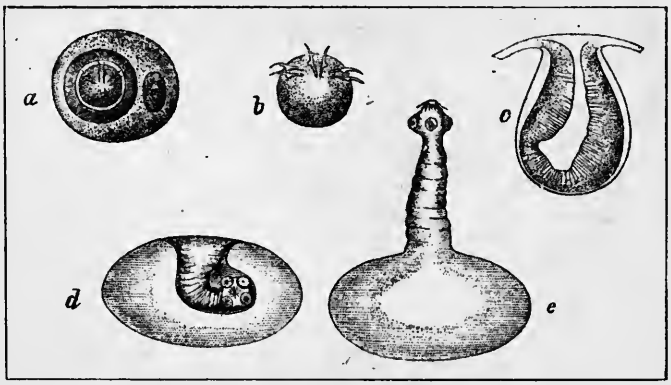


FIG. 88.—Tænia solium. *a*, Ovum: *b*, free embryo; *c*, invagination, at the bottom of which the head forms: *d*, invaginated cysticercus: *e*, evaginated cysticercus. (From Brumpt.)

kind at the Johns Hopkins Hospital. The patient, a laboring man, had never worked as a butcher or a cook, and never had a tapeworm. The cysticercus nodules, which were situated between the skin and the fascia, were very numerous, seventy-five being counted in one day. One of these nodules was removed for examination, and was shown to be referable to the cysticercus of Tænia solium. The only subjective complaints in this case were pains and stiffness in the arms and legs. The individual cysticercus was elliptical or roundish in form, measuring from 1 to 10 mm. in diameter. In its interior the characteristic hooklets were seen (Fig. 88).

Hymenolepis nana (v. Siebold): *syn.*, Tænia nana (v. Sieb.); T. ægyptiaca (Bilharz). This parasite (Fig. 89) seems to be the most common tapeworm of Italy and Egypt. It has also been seen in

Buenos Ayres, in Bangkok, Siam, and a few isolated cases have been reported in England and in Germany. In the United States the

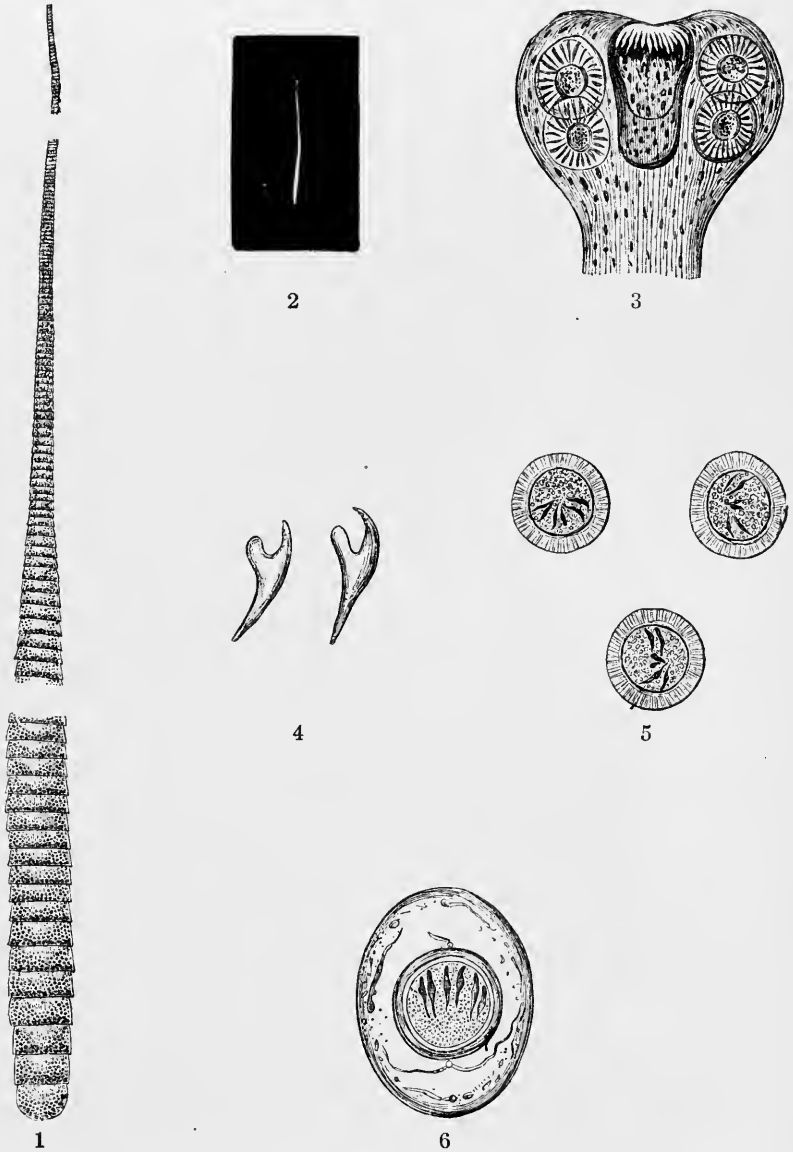


FIG. 89.—Hymenolepis nana: 1, body; 2, natural size; 3, head; 4, hooklets; 5, eggs; 6, egg magnified 600 times. (From Mosler.)

parasite seems to be not at all uncommon, but has probably been overlooked in many cases. Stiles states that in his laboratory eighteen

cases have been diagnosticated within a year (1902). It is found especially in young people, and often causes severe nervous symptoms. It is only 8 to 25 mm. long and 0.5 mm. broad. The head is ball-shaped and provided with four suckers and a rostellum, bearing twenty-four to twenty-eight hooklets arranged in a single row along its anterior edge. The individual segments are of a yellowish color and about four times as broad as long. The uterus is oblong and contains numerous ova. These are colorless, elliptical and provided with an outer and an inner envelope, the diameter of the former measuring about 68μ, that of the latter 29 to 30μ. In the interior of the ovum the hooklets of the embryo may be seen, measuring 15 to 16μ in length. The ova are usually surrounded by the vitelline membrane. The number of worms which may at times be found in the digestive tract is most astonishing; 5000 and even more have been counted on several occasions. The cysticercus stage occurs in snails, which are frequently eaten raw in Egypt, Italy, Belgium and France. Auto-infection readily occurs. The young worms on emerging from the ovum in the intestinal canal, lodge in the villi of the intestinal mucosa. In about sixty-two hours they mature, break into the intestinal lumen and attach themselves to the intestinal wall. The scolex alone develops; by budding this gives rise to the worm proper which matures in two weeks. Tænia nana has been identified with the Tænia murina of mice, rats and other rodents. In doubtful cases the eggs should be looked for; they are readily seen with a low power (Plate XIX).

Hymenolepis diminuta, Rudolphi: *syn.*, Tænia flavapunctata (Weinland); Tænia minima (Grassi); Tænia varesina (Parona); Tænia leptocephala (Creplin). Tænia diminuta was first described in man by Leidy, Grassi, and Parona. It measures 20 to 60 mm. in length, and is armed with two suckers, but is without a rostellum. The ova resemble those of Tænia solium. The cysticercus occurs in certain caterpillars and cocoons. In man it has been found in only six instances.

Dipylidium caninum, Linné: *syn.*, Tænia canina (Linné); Tænia moniliformis (Pallas); Tænia cucumerina (Bloch); Tænia elliptica (Batsch). The parasite is found almost exclusively in children; infection occurs through dogs and cats. In the United States the disease is apparently rare. The only case reported is that of Stiles. The total number of cases recorded in 1912 was 66. The larval form is found in lice and fleas. The worm itself measures from 15 to 35 cm. in length. The head is small, globular; the rostellum club-shaped with 3 or 4 transverse rows of hooks (about 60 in number) of rose-thorn form; anterior hooks 15μ, posterior hooks 6μ; suckers relatively large, rather elliptical. Segments 80 to 120 in number; gravid segments 8 to 11 mm. long, 1.5 to 3 mm. broad; often reddish brown in color. Genital pores at equator or in posterior half of segment; uterus forms egg capsules, each containing from eight

to twenty eggs; eggs globular, 43 to 50μ in diameter. The ova contain embryos already armed with hooklets (Stiles). In diagnosis, Stiles suggests that search be made in the feces for the peculiar elongated elliptical tapeworm segments (Fig. 90). Microscopic examination of the feces for eggs is less certain than in cases of infection with Tænia saginata, Tænia solium, or Dibothriocephalus

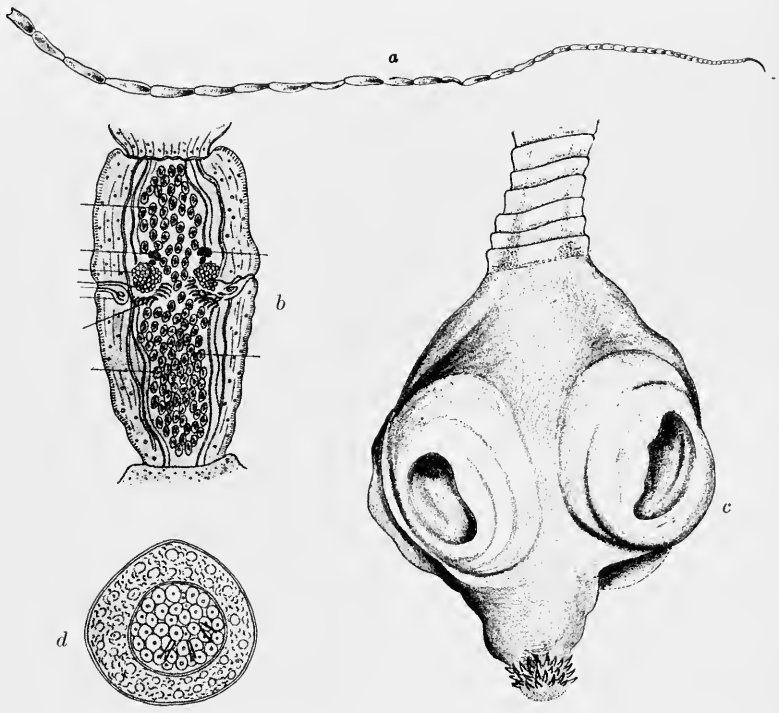


FIG. 90.—*a*, Dipylidium caninum (taken from Stiles); *b*, gravid segment (after Diamare); *c*, head, showing four rows of rose-thorn hooks on the rostellum and four unarmed suckers (Stiles); *d*, egg, showing six hooks of the embryo (Stiles).

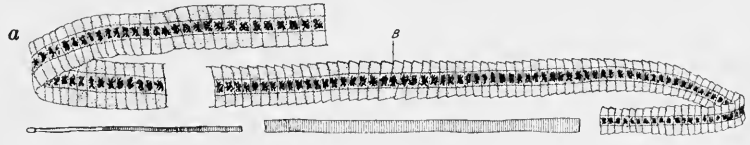


FIG. 91.—Bothriocephalus latus: *a*, *b*, twin segments. (Wilson.)

latus, since Dipylidium is much smaller and less prolific than any of these three forms. In one case reported by Lins 208 parasites were passed by one patient following treatment.

Tænia africana (v. Linstow).—This parasite has been found in two instances, in the case of two native soldiers at Nyasa Lake. Like

the scolex of Tænia saginata, that of the present species is devoid of hooklets. Its length is about 1.4 m.; the number of segments about 600. They are all much broader than long. The uterus consists of a main portion running fore and aft, from which from 15 to 24 side branches issue, which do not branch dichotomously and are so closely packed that they cannot be recognized with the naked eye.

Tænia madagascariensis (Grenet).—This parasite has been found in Madagascar, in Mauritius, in Bangkok, and in a Demarara Indian. The worm attains a length of from 25 to 30 cm., and is composed of from 500 to 600 trapezoid segments. The rostellum is surrounded by a double row of minute hooklets. The suckers are round and quite large. Blanchard suggests that the cockroach may be its intermediary host.

Dibothriocephalus latus, Linné, Lueke: *syn.*, Bothriocephalus latus, (Bremser); Tænia lata (Linné); Dibothrium latum (Rudolphi) (see Fig. 91). This worm is usually 5 to 10 m. long and of a reddish-gray color. Longer specimens, however, may also be encountered. In Wilson's case 82 feet of segments were obtained from two worms, so that the length of each, supposing both to have been of the same size, must have been more than 40 feet. The head is almond-shaped, and upon its flat surfaces two distinct grooves can be discerned, which probably act as suckers. It measures 2 to 3 mm. in length by 1 mm. in breadth. The neck is very short and passes at once into the body segments. Adjacent segments can often be distinguished only by means of the recurrence of the sexual apparatus, which appears regularly in spite of the imperfect individualization of the segments. The ripe segments are almost square in form with the genital apparatus opening in the median line. The fully developed segments measure 2.5 to 4.5 mm. in length by 8 to 14 mm. in breadth. The total number of segments may far exceed 3000. The frequent occurrence of imperfect and abortive types of twin segments may be considered an almost distinctive feature of the bothriocephalus family (Wilson). The uterus presents 4 to 6 convolutions on each side, which become especially distinct when the segments are placed in water or are exposed to the air. A rosette-like appearance is then noted, which is quite characteristic (Fig. 85). The rosette deepens in color in proportion to the number of ova which the uterus contains, and toward the tail of the parasite, from the segments of which many or all the eggs have been discharged, the rosette tends to become light in color, and may indeed appear whiter than the surrounding parenchyma. The eggs (Fig. 92) are oval, 60 to 70μ long and about 45μ broad; they are inclosed in a brown envelope, at the anterior end of which a little lid can be recognized. Their contents consist of protoplasmic spherules, all of about the same size, which are lighter in color in the centre than at the periphery. In infected individuals they are constantly found in the stools.

The larvæ have been found in various fresh-water fishes, such as the perch, the ling, the turbot, in various members of the trout family, but they are most commonly encountered in the pike. It is thus readily understood why the parasite is most common in lake regions, as in Switzerland, northern Russia, southern Scandinavia, and northern Italy. It is seldom seen in middle Germany, but is so common in Ireland that Cobbold named it the Irish tapeworm. Outside of Europe it is most common in Japan. In the United States a few imported cases have been observed by Walker and Leidy, Packard, Hageestam, Riesman, Stengel, McFarland, and Wilson.

Multiple infection has been repeatedly mentioned. Böttcher notes a case in which 100 worms were found; Roux and Eichhorst both speak of cases with 90, Heller of one with 38, and in Wilson's case 2 were undoubtedly present. When more than 1 occurs the growth

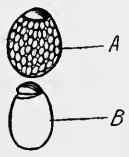


FIG. 92

FIG. 93

FIGS. 92 and 93.—Eggs and plerocercoid. (Braun.)

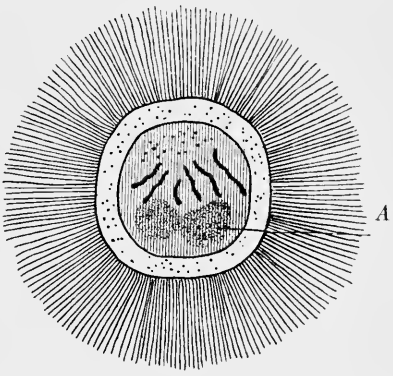


FIG. 94.—Embryo with cilia and hooklets of Dibothriocephalus latus. (Leuckart and Braun.)

of the individual is impeded, and small specimens are then usually seen (three to five feet or more). Clinically the parasite is of special interest, as its presence in a certain percentage of cases is associated with the clinical picture of pernicious anemia; in others, however, no deleterious effect upon the red corpuscles is noted, although several worms may be present in the intestinal tract.

Besides in man, the worm has been encountered in the dog, cat, the seal, and in some water birds. The ovum after being discharged in the feces, during a variable period of incubation in the water develops into the onchosphere, a ciliated larva with six hooklets (Fig. 94). The larva is then liberated from the ovum by passing through the lidded end, and by means of its cilia moves rapidly through the water. If not eaten by fish it dies; otherwise it develops into the bothriocephalus measle, the plerocercoid (Fig. 93), which has both head and tail. Infection of man then occurs when such

fish are eaten either raw or but partly cooked. In man the cysticercus stage has not been observed.

Diplogonaporus grandis (Blanchard): *syn.*, Krabbea grandis (Bl.). This parasite has been observed in only one instance—in Japan. It is said to resemble certain bothriocephali which are found in seals. The genital organs are double in each segment. The vulva and uterus opens ventrally. The worm attains a length of 10 m. with a breadth of 2 cm.

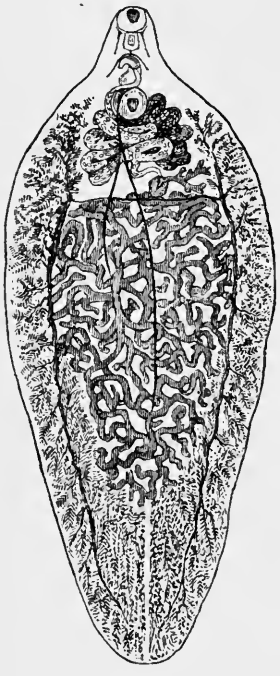


Fig. 95.—Distoma hepaticum, with male and female genital apparatus. (From Ziegler, after Leuckart.)

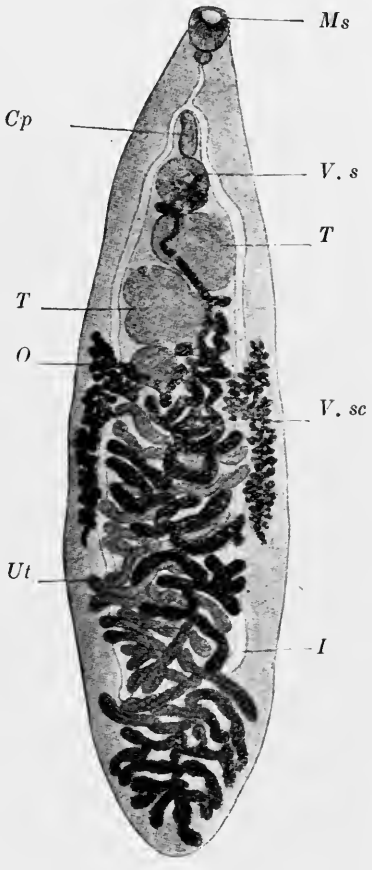


Fig. 96.—Dicrocœlium (Distoma) lanceolatum, Stil. and Hass *V. s*, ventral sucker; *Cp*, pouch of cirrus; *I*, intestinal furcations; *V. sc*, vitelline sacs; *T*, testicles; *O*, ovarium; *Ms*, oval sucker; *Ut*, uterus.

Trematodes.—The various forms of distoma which belong to this order are essentially hepatic parasites, and rarely occur in the feces.

Fasciola hepatica (Linné): *syn.*, Distomum hepaticum (Retz) (Fig. 95). This, the most common liver fluke, is 28 mm. long and 12

mm. broad; it is formed like a leaf. The leaf is provided with a sucker, and a second sucker may be found at its ventral surface. Between the two the genital opening is located, leading into a skein-shaped uterus. The eggs are oval, measuring 0.13 mm. in length and 0.08 mm. in breadth, the anterior end being provided with a lid; their color is brown. In the United States the organism is practically unknown, while in Germany it is most common in sheep. In the human being it is rare in both countries. It occurs in cattle, sheep, swine, cats, rabbits, etc. Infection occurs through a small snail, the Linnæus minutus, which is found, in Germany especially, upon watercress.

Dicrocœlium lanceolatum, Stil. and Hass; Distoma lanceolatum, Mehlis. This parasite has been found in seven cases only (Germany, Bohemia, Italy, France and Egypt) (Fig. 96). It is much smaller than Distoma hepaticum, measuring 8 to 9 mm. in length by 2 to 3.3. mm. in breadth. It is lancet-shaped, tapering toward the head end, but otherwise closely resembles Distoma hepaticum. The ova are 0.04 mm. long, 0.03 mm. broad, and contain fully developed embryos. In cattle, sheep, and hogs the organism is quite common.

Fasciolopsis buski (Lankester): *syn.*, Distomum buski (Lankester); Distoma crassum (Busk) *nec* (v. Siebold); Distoma cranium (Busk). The parasite has been observed in seven cases (China, Sumatra, the Straits Settlements, Assam, and India). An imported case has been described in the United States (Moore). It is the largest distoma occurring in man, measuring over an inch in length. It probably inhabits the upper portion of the intestine, and may give rise to attacks of recurring diarrhea and other signs of intestinal irritation. Infection probably occurs through certain fishes and oysters, with certain snails as intermediary hosts.

Distomum rathonisi (Poirier) is closely related to, but not identical with, the parasite just described. Three cases have been described (China and North Borneo).

Opisthorchis felineus (Rivolta): *syn.*, Distoma conus (Gurlt); Distoma sibiricum (Winigradoff). This parasite was found in Tomsk, by Winigradoff, in eight autopsies out of one hundred and twenty-four. It was the most common parasite that came under observation. Askanazy reports two cases of infection from eastern Prussia, in which the eggs were found in the stools. In one of the cases, which came to section, more than one hundred organisms were found in the biliary passages. Its length may reach 13 mm. The ova are 0.026 to 0.038 mm. long and 0.010 to 0.022 mm. broad. The intestine is simple and extends to the posterior extremity of the body. Its surface is smooth.

Opisthorchis sinensis (Cobbold): *syn.*, Distoma spatulatum (R. Leuckart); Distoma sinense (Cobbold); Distoma endemicum (Balz); Distoma japonicum (Blanchard). It has been observed in India,

Mauritius, Corea, Formosa, China, Tonkin, and Japan, and it appears that in the two last-named countries it is quite common. It inhabits the biliary passages and gall-bladder. It is distinctly pathogenic. The ova may be found in the stools. The parasite possibly also occurs in cats. The intermediary host is not definitely known; it may be some fresh-water mollusk. It is about 11.75 mm. long and 2 to 2.75 mm. broad. The living parasite is of a reddish

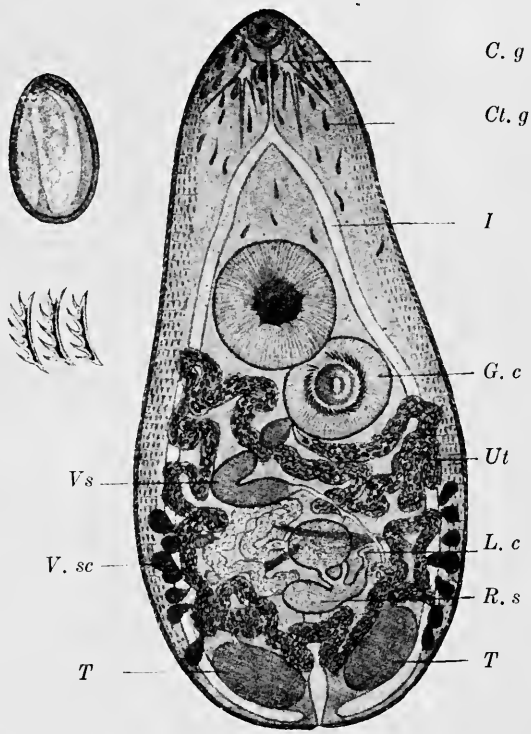


FIG. 97.—Cotylogonimus (Distoma) heterophyes. × 53 (v. Sieb.) *C. g*, cerebral ganglion *I*, intestinal branches; *Ct. g*, cuticular glands; *V. sc*, vitelline sacs; *G. c*, genital cup; *T*, testes the excretory bladder between them; *L. c*, Laurer's canal; *R. s*, receptaculum seminis, with the ovarium in front of it; *Ut*, uterus; *Vs*, vesicula seminalis. On the left side above an egg × 700 is depicted, and below it three chitinous rodlets from the genital cup. × 700. (Looss.)

color and translucent, so that it is possible to distinguish all its interior organs. The ova measure 0.028 to 0.03 mm. in length by 0.016 to 0.017 mm. in breadth, and are inclosed in a colorless envelope.

Other parasites belonging to this order are **Opisthorchis noverca** (nov. nom.): *syn.*, Distoma conjunctum (Lewis and Cunningham); **Cotylogonimus heterophyes** (v. Siebold): *syn.*, Distoma heterophyes,

and **Amphistomum hominis** (Lewis and McConnell). The last named appears to be common in elephants, and has been encountered in natives of Assam, in two Indians in Calcutta, and in an East Indian immigrant in British Guiana. It is quite small, measuring from 5 to 8 mm. in length by 3 to 4 mm. in breadth, and is characterized by the large size of its posterior suckers.

Cotylogonimus heterophyes is the smallest distoma, so far as we know, which is found in man. It occurs in Egypt, and is thought to be innocuous (Fig. 97).

Opisthorchis noverca was discovered in an East Indian. Its surface is covered with minute spicules. It is not of much pathological importance (Fig. 98).

Schistosomidæ, Looss; **Schistosoma japonicum** (Katsurada): *syn.*, Sch. cattoi (Blanchard). This parasite seems to be fairly common in certain districts of Japan (Yamauashi, Hiroshima, and Saga) as also in China, causing diarrhea, anemia, occasionally fever, and in some instances death. Isolated cases have been observed in the Philippines, in England and the United States, those in the last two countries evidently having been imported. The adult worm occurs in the smaller mesenteric bloodvessels. In a general way it resembles the Schistosomum hæmatobium. It is smaller, however, and the posterior sucker is larger than the anterior one; the integument of the male is smooth. The eggs are found especially in the walls of the intestinal tract and in the liver, but are also found quite commonly in the feces. They resemble the ova of the hookworm in size, shape, and general appearance, but contain a ciliated embryo which may develop in the intestinal canal before the eggs are evacuated. They are smaller than the eggs of Schistosoma hæmatobium and lack the lateral spine of the latter. They measure on an average 55μ in length and 31μ in breadth, when obtained from the uterus of the female, while somewhat larger ones are seen in the feces.

Nemathelminthes.—These comprise the Nematodes, the Gordiides and the Acanthocephali, and of these in turn the Nematodes are represented in the intestinal tract of man. They include the Ascarides the Strongyloides, the Trichotrachelides and the Anguillulides.

Ascaris lumbricoides, Linné (Fig. 100), is the cylindrically shaped worm so commonly seen in children and in the insane. The head consists of three projections or lips, which are provided with suckers and fine teeth. The male measures about 215 mm., the female about 400 mm. in length. The tail end of the male is rolled up on its ventral surface like a hook, and is provided with papillæ. The genital aperture of the female is situated directly behind the anterior third of the body. The eggs are yellowish brown in color, almost round, and measure 0.06 mm. by 0.07 mm. in size; they are surrounded by an irregular albuminous envelope, which is covered with

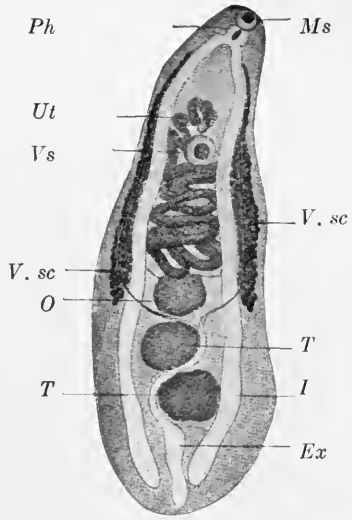


FIG. 98.—Distoma. Opisthorchis noverca, Cobb (*nec* Lewis and Crum; *nec* McConnell), from Canis fulvus (Cobbold): *Vs*, ventral sucker; *I*, intestine; *V sc*, vitelline sacs; *Ex*, excretory bladder; *T*, testes; *O*, ovary; *Ms*, oral sucker; *Ph*, pharynx; *Ut*, uterus.

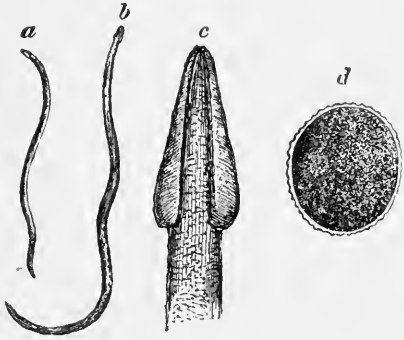


FIG. 99.—Ascaris mystax: *a*, male; *b*, female; *c*, head; *d*, egg. (v. Jaksch.)

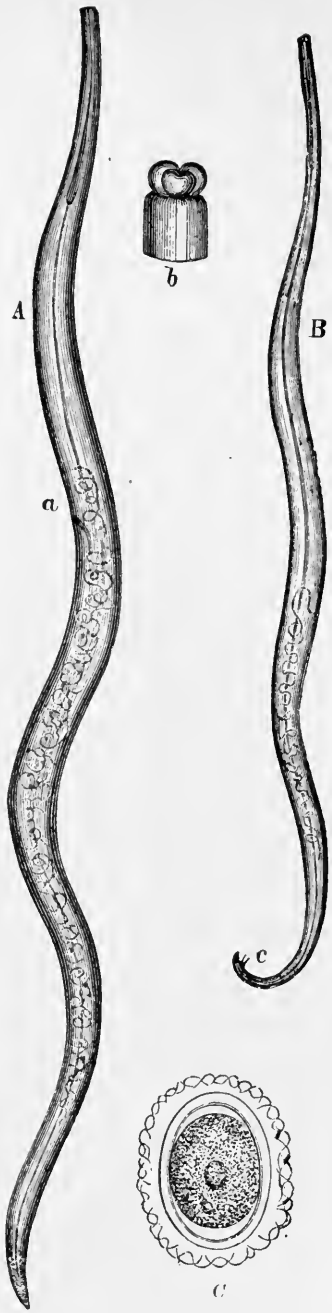


FIG. 100.—Ascaris lumbricoides: *A*, female; *B*, male; *C*, egg. At *a*, the female genital opening; *b*, the enlarged cephalic extremity. with its three lips; *c*, the male spicules, (After Perlo, from Ziegler.)

a tough shell; the contents are coarsely granular[1] (Plate XIX). At the time of defecation the eggs contain but a single cell and no embryos, as in the case of oxyuris, for example, and for this reason a self-infection is not possible.

Outside of the body the embryo develops either in water or in damp earth in from thirty to forty days if the weather is warm, while at lower temperatures the process may extend over several months. The developing ovum is quite resistant to heat (42° C.) as well as cold. After being swallowed the embryo escapes from the surrounding capsule and develops within five or six weeks into the adult worm.

There is thus no intermediary host.

Infection of necessity occurs through the ingestion of egg-bearing dirt, either as such (by children, or the insane) or attached to fruits or vegetables, consumed in uncooked form. Children and young animals seem to offer a more favorable soil for their development than adults.

The number of worms which may infect an individual is usually small (5 or 6), but exceptional cases have been reported where as many as 1000 were found. One instance even is mentioned, where a child had passed 5000 worms in the course of a few years.

The number of eggs which one encounters, even though only a couple of worms be present in the intestinal tract, is usually quite large, so that it is only necessary to emulsify a bit of fresh material, and to mount a single drop in order to find a dozen or more in that quantity.

Ascaris lumbricoides is found in all countries, and also infests the pig and the ox.

Ascaris canis (Werner): *syn.*, Lumbricus canis (Werner); Ascaris marginata (Rudolphi); Ascaris alata (Bellingham); Ascaris mystax (Leder) (Fig. 99). This worm is smaller and thinner than Ascaris lumbricoides, but otherwise very similar. The head is pointed and provided with wing-like projections which constitute the main point of difference between the two. The male measures 45 to 60 mm. in length, the female 110 to 120 mm. Its ova are round, larger than those of Ascaris lumbricoides, and enclosed in a membrane which is covered with numerous small depressions. The worm is common in dogs and cats, but very rare in man. Only eleven cases have been reported (England, Germany, Denmark, North America) (1912).

Ascaris maritima, Leuckart, also belongs to this class. It has been observed in only one case—in Greenland.

Ascaris texana (Smith-Goeth).—A supposedly new species, which has been found in a single instance in Texas. The male has not yet

[1] A hermaphroditic egg has also been described which is oval in form with grayish granular contents.

been described. The females measured from 58 to 60 mm. by 1 to 1.5 mm. and the eggs 40 by 60μ.

Oxyuris vermicularis, Bremser: *syn.*, Ascaris vermicularis (Linné); Ascaris græcorum (Pallas) (Figs. 101, 102, and 103). The male is 4 mm., the female 10 mm. long. At the head three lip-like projections with lateral cuticular thickenings may be seen. The tail of the

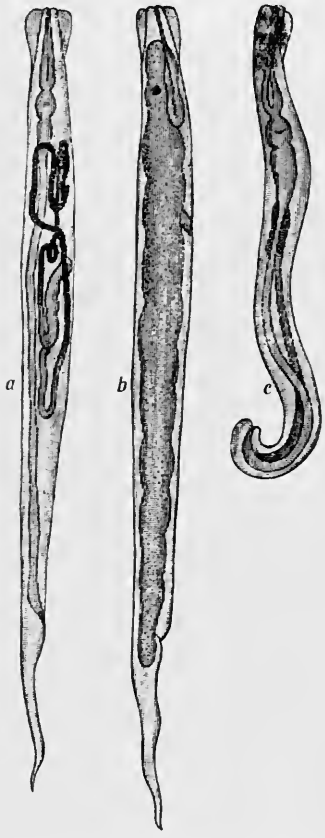


FIG. 101.—Oxyuris vermicularis: *a*, sexually mature female; *b*, female filled with eggs; *c*, male. Magnification, 10. (After Heller, from Ziegler.)

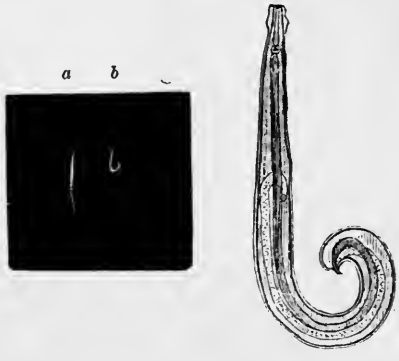


FIG. 102.—*1*. Oxyuris vermicularis: *a*, male; *b*, female; natural size. *2*. Magnified.

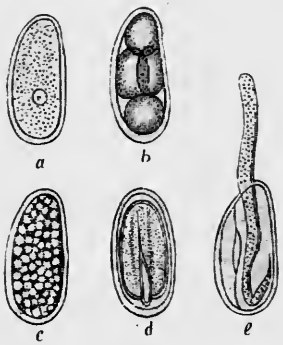


FIG. 103.—Eggs of Oxyuris vermicularis in various stages of development: *a*, *b*, *c*, division of the yolk: *d*, tadpole-like embryo; *e*, worm-shaped embryo. Magnification, 250. (After Zenker and Heller, from Ziegler.)

male is provided with six pairs of papillæ and the female with two uteri. The eggs measure 50 to 54μ in length by 20 to 27μ in breadth; they are asymmetrical, and covered with a membrane showing a double or triple contour; in the interior, which is coarsely granular, the embryos are contained (Plate XIX).

The female worm lives in the cecum, but after impregnation

FIG. 105.—Ankylostoma duodenale, male and female. Natural size. (From Mosler.)

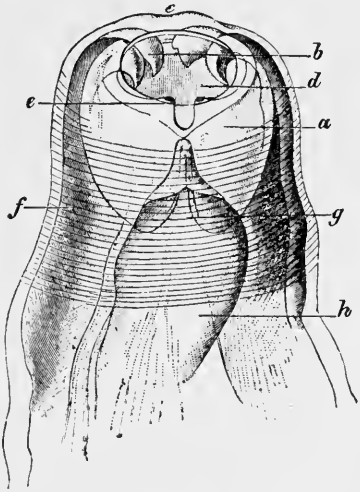


FIG. 106.—Head of Ankylostoma duodenale: *a*, buccal capsule; *b*, teeth of capsule; *c*, teeth of dorsal margin; *d*, oral cavity; *e*, ventral prominence; *f*, muscle layer; *g*, dorsal groove; *h*, esophagus. (After Schulthess, from Ziegler.)

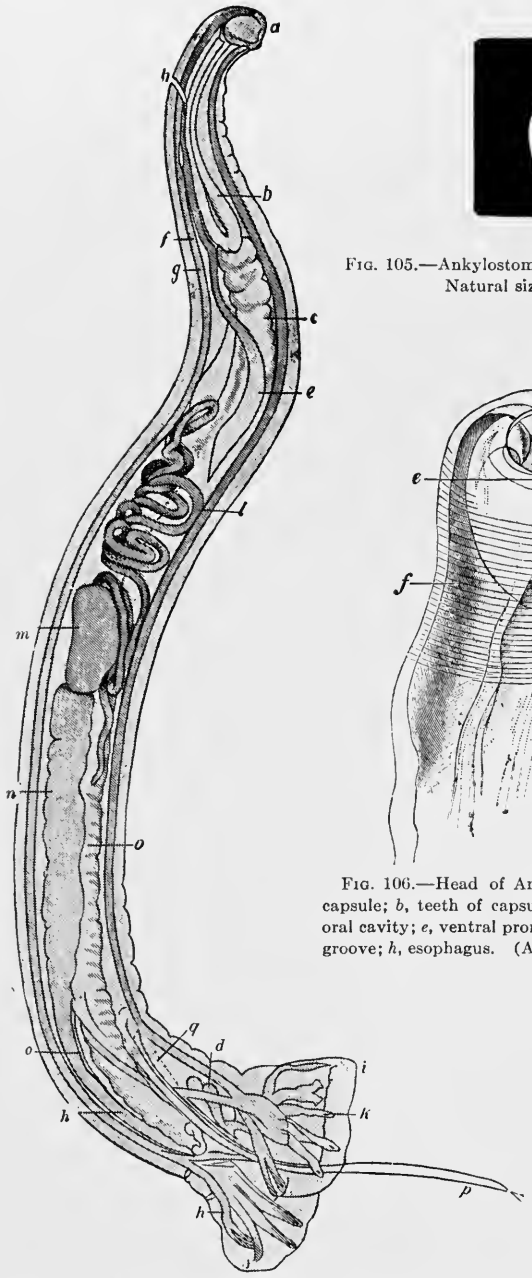


FIG. 104.—Male Ankylostoma duodenale: *a*, head; *b*, esophagus; *c*, gut; *d*, anal glands; *e*, cervical glands; *f*, skin; *g*, muscular layer; *h*, excretory pore; *i*, trilobed bursa; *k*, ribs of bursa; *l*, seminal duct; *m*, vesicula seminalis; *n*, ductus ejaculatorius; *o*, its groove; *p*, penis; *q*, penile sheath. Magnification, 20. (After Schulthess, from Ziegler.)

travels downward to the rectum. Here it causes most annoying symptoms, which are especially distressing at night, when the organism emerges from the anus. In doubtful cases of pruritus ani et vulvæ an examination of the feces should be made for this parasite. The ova themselves do not occur in the feces, and the diagnosis is usually made by finding the worms either about the anus or in the stools.

Infection occurs through the ingestion of egg-bearing dirt taken either as such or occurring in the form of dust on fruits and vegetables.

Auto-infection frequently occurs. This is easy to understand, if we bear in mind the readiness with which the fingers or finger-nails may become bearers of eggs or even of female worms during the process of anal scratching.

The parasite is a very common one, but scarcely as much so in the United States as in certain other countries. Heller, in northern Germany, claims to have found 18.8 per cent. of all males, 21.1 per cent. of all females and 33.8 per cent. of all children infected.

The Strongyloides.—Two types are known to infect the human being, viz., a European type and an Africo-Asian type. The former was formerly spoken of as the old-world hook worm, in contradistinction to the one which was first discovered in the United States by Stiles, and regarded as a type peculiar to the new world. Late researches have shown, however, that the latter was unquestionably introduced into the new world (North, Central, and South America) from Africa along the slave trade routes, and hence hardly merits the name of the new-world hook worm.

The European type is known as **Uncinaria duodenalis** (Roilliet); *syn.*, Ankylostomum duodenale (Dubini), Strongylus quadridentatus (v. Siebold), Dochmius ankylostomum (Molin), Sclerastoma duodenale (Leuckart). It has been found in Italy, Germany, Switzerland, Belgium, France, etc.

The American type has been named **Uncinaria americana** (Stiles); syn. Necator americanus (Stiles), Ankylostoma americanum (Verdun). It occurs throughout the sand belt of the South (the Carolinas, Georgia, Florida, Alabama, Texas), in the islands of the West Indies and Brazil, among the pygmies of Central Africa, the negroes of Kamerun, of Oubangui, of the Congo-Nile, French Guiana, as well as the inhabitants of Annam (Asia).

Infection occurs very largely through the skin and perhaps altogether so. C. A. Smith insists that uncinariasis exists in all cases in which "ground itch" has occurred within eight years, and that the disease is rarely if ever present in those who have not had ground itch within that time.

From a pathological standpoint the parasite is of special interest, as its presence may give rise to severe and fatal anemia. Griesinger was the first to point out that the so-called Egyptian chlorosis

is produced by this organism. Subsequently it was shown that the same parasite was responsible for the anemia which developed among the workers on the St. Gothard Tunnel, and which is common among the brickmakers in certain districts of Germany (about Bonn and Cologne). In this country the anemia of the dirt-eaters has

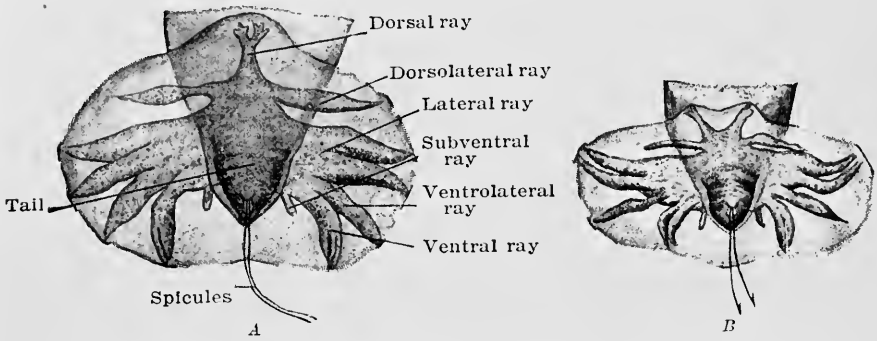


Fig. 107.—*A*, caudal bursa and tail of male Uncinaria duodenalis; *B*, caudal bursa and tail of male Uncinaria americana. Drawn to scale to show difference in size. (After Allen J. Smith.)

long been known in the South, and has been generally attributed to the peculiar habit. Its real cause is now manifest. In Porto Rico the disease was very common until very recently and responsible for much of the severe anemia which was so frequent among the natives. In Germany, France, and Belgium the mining districts have become extensively infected and the eradication of the disease a serious problem. All through the southern sand belt of the United States it is responsible for a great deal of the existing anemia which formerly was ascribed to malaria almost exclusively. Outside of man the parasite is not uncommon in dogs, cattle, and sheep.

Fig. 108.—Copulating hook worms from dog. (Bock and Bass.)

The following description applies to the European hook worm: The male is 6 to 11.5 mm. long, the female 10 to 18 mm. The head, which tapers somewhat, is turned toward the back; the mouth capsule is hollowed out and surrounded by 4 teeth; the tail of the male forms a 3-lobed bursa, while that of the female tapers conically; the genital opening is behind the middle of the body (Figs. 104 and 107). Its eggs have an oval form and a smooth surface, measuring from 50 to 60μ in length by 30 to 40μ in breadth. In their interior two or three segmenting bodies are found, which rapidly develop outside of the

human body, so that after twenty-four to forty-eight hours embryos may be found in the same feces in which the eggs were observed, or fully developed ova may be found after allowing the feces to stand for only a few hours (Plate XIX). When allowed to dry, the young parasites become encysted, but after remaining so even for from one to two weeks they are capable of infecting. A second host for its cycle of development is, according to Leichtenstern, not necessary.

The Africo-American worm can hardly be distinguished from the European type with the naked eye. The differences lie in the construction of the buccal capsule and in the copulatory bursa of the

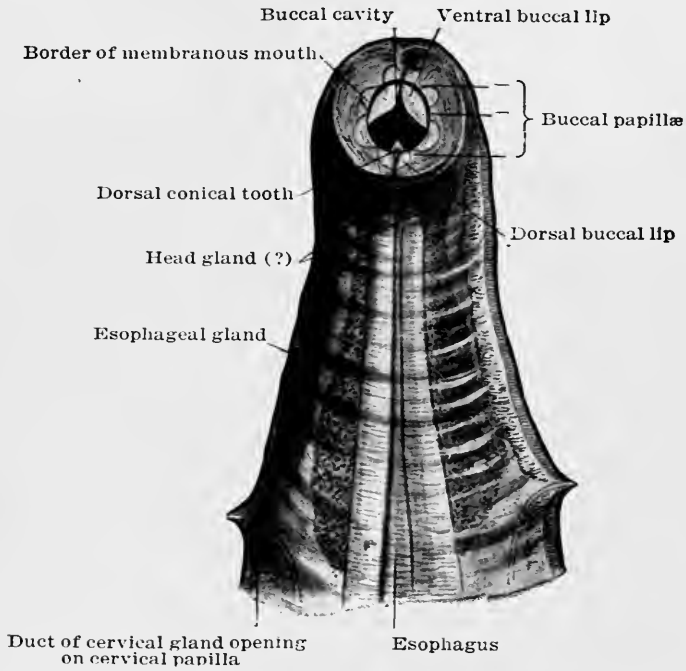


Fig. 109.—Dorsal aspect of head end of Uncinaria americana. (After Allen J. Smith.)

male. In the place of the four ventral hooks which we see in the European type we here find two broad lips and a blunt, conical, dorsal tooth instead of two (Figs. 106 and 109). The differences in the bursæ are shown in the accompanying figure (Fig. 107). In the African type there is a dorsal bilobed lobe; the posterior ray is split to its base, each branch being bifid instead of trifid as in the European form.

The habitat of the adult worm is the jejunum. It is rarely found in the feces. Its eggs, however, are common, and should be looked for in every case of anemia the cause of which is not manifest, especially in miners, tunnel-workers, brickmakers, dirt-eaters, etc. Any

specimen of fecal material will answer, as a rule, but it is best to procure a thin stool, as after a purge. It is then merely necessary to mount drops on slides and to examine the covered specimens with a low power; a Bausch & Lomb $\frac{2}{3}$ is quite sufficient. A mental picture of the size of the eggs should be made, for I have known it to occur that an observer saw the eggs, but did not recognize them as such. Once seen, they are easily recognized again.

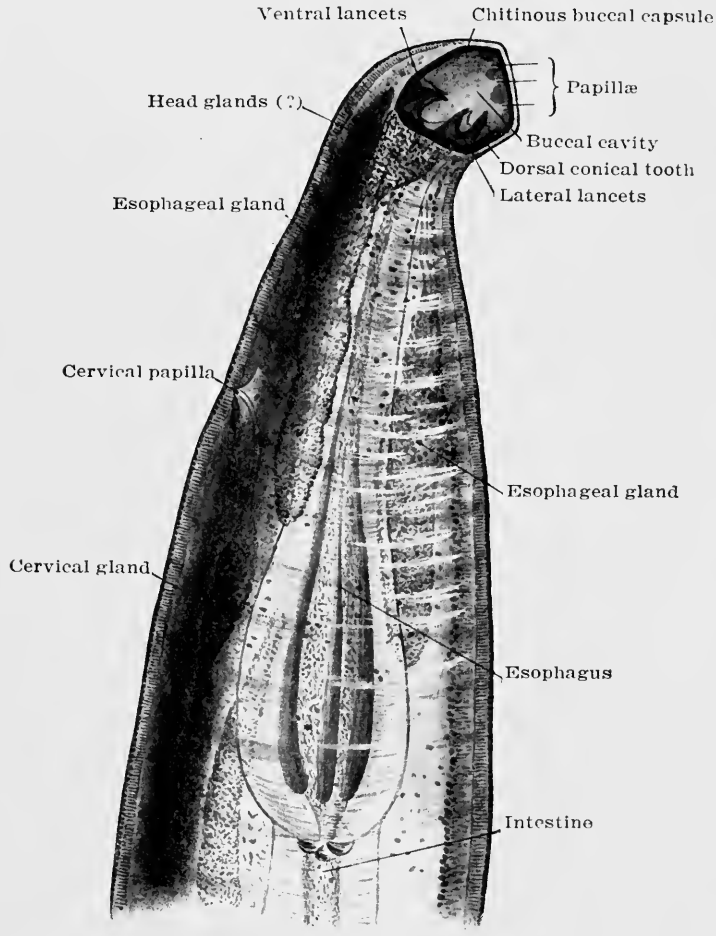


Fig. 110.—Lateral aspect of head end of Uncinaria americana. (After Allen J. Smith.)

The ova of the Africo-American worm are somewhat longer than those of the European type, measuring 70μ in length by 40μ in breadth.

To hatch the eggs artificially, Smith recommends to mix the fecal material with a small amount of soil in a Petri dish, using a sufficient

amount of water for the purpose. There should be just sufficient moisture to keep the soil damp. If there is too much the cover is left off for an hour or so. Every two or three days a few drops of water are added to replenish the moisture. Under favorable conditions in this respect all the eggs will hatch within twenty-four hours; otherwise several days will elapse. In such cultures the larvæ will remain alive for three or four months and can be observed with a $\frac{2}{3}$ in the inverted dish.

FIG. 111.—*A*, dorsal view of head end of Uncinaria duodenalis; *B*, dorsal view of head end of Uncinaria americana. Drawn to scale to show difference in size. (After Allen J. Smith.)

To give an idea of the enormous number of eggs which may be passed by a single hook-worm patient it will suffice to refer to Bass' calculations, according to which a patient with a moderate infection passed 1,700,000 eggs a day. The same writer speaks of from 1000 to 4000 worms as representing a severe infection.

The accompanying diagram illustrates in brief the life cycle of the organism: (taken from Brumpt):

OVUM.

Passed into the intestinal canal of man, containing two, four, or eight segments (blastomeres). Outside of the body there emerges in twenty-four hours

A RHABDITIFORM LARVA (1ST STAGE)
This moults on the third day: on the fifth the pharyngeal swellings disappear and a second moult occurs with the formation of a capsule about

↓

THE ENCYSTED STRONGYLOID LARVA (2D STAGE)
This reaches the digestive canal of man through the skin (more rarely the mouth), becomes free and constitutes

↓

THE LARVA DEVOID OF A BUCCAL CAPSULE (3D STAGE)
Between the fifth and seventh day another moult occurs (3d stage) and there results

↓

A LARVA WITH A TEMPORARY BUCCAL CAPSULE (4TH STAGE)
About the fifteenth day a fourth moult takes place, giving rise to

↓

A LARVA WITH FULLY DEVELOPED BUCCAL CAPSULE (5TH AND LAST STAGE)
This grows and in from three to four weeks develops into

↓

ADULT SEXUAL FORMS ♂ AND ♀

↓

OVA

Trichocephalus trichiurus (Linné): *syn.*, Ascaris trichiura (L.); Trichocephalus hominis (Schwank); Trichocephalus dispar (Rudolphi). This parasite, which belongs to the family *Trichotrachelides*, is formed like a whip, the last end being the head end, while the tail end is very much thicker. The male measures 46 mm. and the female 50 mm. in length. The eggs are brownish in color, measuring 22 by 50μ in size, and present a doubly contoured shell, with a depression at each end, closed by a lid. The contents are coarsely granular. The organism is said to be the most widely distributed intestinal parasite, occurring in Europe, North America, Asia, Africa, and Australia. Its habitat is the cecum and the appendix, while it is less frequently found in the colon. The living worm is only rarely found in the feces, while the eggs can be readily demonstrated, attracting attention at once by their color and graceful outline (Fig. 112 and Plate XIX). As the ovum which is evacuated in the feces contains only a single cell auto-infection does not occur. The embryo develops very slowly outside of the body.

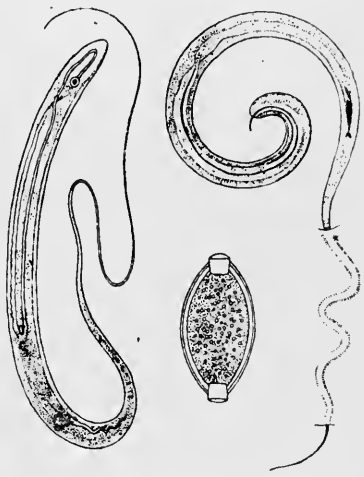


FIG. 112.—Trichocephalus trichiurus. On the left, male; on the right, female with the anterior extremity embedded in the mucous membrane of the intestine. Below, egg.

Trichinella spiralis (Owen): *syn.*, Trichina spiralis (Owen). The male measures 1.5 mm. in length by 0.04 mm. in breadth, and is provided with four papillæ between the conical lips. The female is 3 mm. long. The uterus is situated nearer the head than the ovary, which opens into it. Fertilization occurs in the small intestine. After this has taken place the females emigrate into the intestinal walls, where they seem to have a special affinity for the lymphadenoid tissue. Here the young embryos, which have meanwhile entered the uterus, are discharged, and largely through the lymphatics enter the general circulation and are thus distributed over the body. They in turn seem to have a special affinity for muscle tissue, where they encyst themselves and where they may be found in enormous numbers. The young worms, of which, on an average, at least 1500 are derived from one mother worm, measure 90 to 100μ in length by 6μ in diameter. With the naked eye the cysts appear as minute little white specks which measure about 0.25 by 0.4 mm. The worms can be rendered easily visible by placing a bit of the tissue in glycerin containing 5 per cent. of acetic acid; after a few minutes it is pressed out between two slides and examined with a low power (Fig. 113).

During the period of their migration they may also be demonstrated in the peripheral blood and have even been found in the cerebrospinal fluid (which see). Diagnosis has been greatly facilitated by the discovery that eosinophilia, usually of high grade, is practically a constant symptom during the acute stage of the disease (see Trichinosis). While it is believed that trichinosis is less common in the United States than in Germany, there can be no doubt that it is much more common than was formerly believed. Many light

Fig. 113.—Trichinella spiralis in muscle.

cases go practically unrecognized, unless a blood examination reveals the existence of eosinophilia. In doubtful cases a small bit of muscle tissue should be removed (from the neighborhood of the tendinous end of the biceps or gastrocnemius) and examined as just described. Infection occurs through the ingestion of infected pork which has not been subjected to a sufficient degree of heat to kill the contained larvæ. Sausage meat seems to be the most dangerous, at least in those countries where this is eaten uncooked.

Strongyloides intestinalis (Bavay): *syn.*, Anguillula intestinalis (Bavay); Anguillula stercoralis (Bavay); Rhabditis stercoralis (Bavay); Leptodera stercoralis (Bavay, Cobbold); Leptodera intestinalis (Bavay, Cobbold); Strongyloides intestinalis (Bavay, Grassi); Pseudorhabditis stercoralis (Bavay, Perroncito); Rhabdonema strongyloides (Leuckart); Rhabdonema intestinale (Bavay, Blanchard).

In the feces of patients infected with the parasite in question the eggs of the mother worm are only rarely found, and the adult worm itself probably never appears unless an anthelmintic has been administered and active catharsis established. Instead we find embryos (rhabditic form) measuring about 330 by 22μ in size. If the stools are kept, uncovered, at a temperature of about 37° C., their larvæ undergo development and reach full growth and sexual differentiation in about five days. The length of the full-grown female is about 1 mm.; its breadth about 0.04 mm. The body is cylindrical, slightly diminishing in size anteriorly, and tapering to a sharp point posteriorly. When the worm retracts forcibly, slight transverse furrows may be seen. The mouth possesses distinct lips and is continuous with a triangular esophagus, which beyond a constriction dilates again into a second ovoid enlargement. The intestine which follows ends in a little protrusion on one side of the body near the base of the tail. A little below the middle of the body, and on the ventral side, is the vulva, which leads to the uterus, extending from the intestinal ventricle to a point near the anus. Here the eggs may be massed in varying numbers. Sometimes the young have actually broken the shell of their eggs and may be seen free in the uterus; but more commonly the ova, on deposition, contain well-formed motile embryos (filariform brood). The male is about one-fifth smaller than the female. The testicle ends at the base of the tail, in two small, horn-like spicules with tapering ends, which are curved inward. These spicules contain canals; they are of equal size and situated symmetrically on a transverse plane. The tail is coiled in the same direction as the spicules, and is half as long as that of the female.

The sexually mature and differentiated forms just described represent the *Anguillula stercoralis* of Bavay. They represent an intermediate generation, developing outside of the body, which forms a link in the chain of development of the mother worm, the *Anguillula intestinalis* (Leuckart).

Ordinarily infection takes place through the larvæ of the sexually differentiated form. These filariform embryos are longer than the rhabditiform brood of Anguillula intestinalis (Fig. 114). They are provided with a cylindrical esophagus descending down to about the middle of the body, and a tail which, instead of terminating in a fine point, is apparently truncated at its extremity. On maturing they give rise to the Anguillula intestinalis, which is encountered

throughout the upper gastro-intestinal tract, especially in the lower part of the duodenum and the upper part of the jejunum, though occasionally they have also been found throughout the entire jejunum and in the upper part of the ileum. On several occasions they have been found in the stomach.

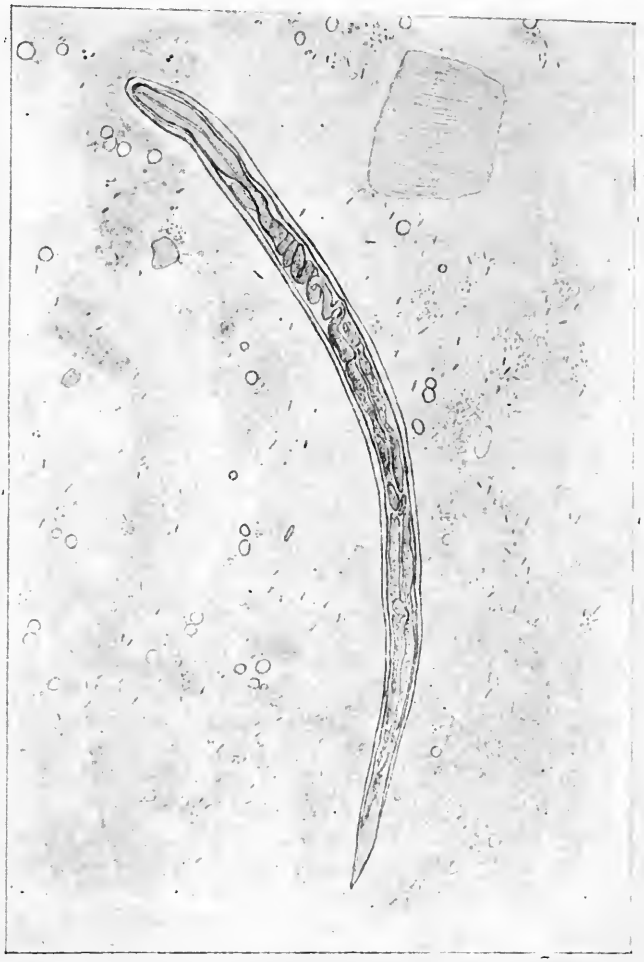


Fig. 114.—Strongyloides embryo (rhabditiform variety). The stool contained many red cells.

Anguillula intestinalis, viz., the parasitic mother worm, is, according to Rovelli, parthenogenetic, while Leuckart expressed the opinion that it might be hermaphroditic. Its length is about 2.2 mm. and its average breadth 0.03 mm. The body tapers a little anteriorly, and terminates posteriorly in a conical tail, the extremity of

which is appreciably rounded and even a trifle dilated. The mouth is without horny armature, and shows three small lips. It opens into a cylindrical esophagus, which occupies about one-fourth of the length of the animal, and shows neither swellings nor striations. The intestine extends nearly to the posterior extremity of the body, but is almost invisible in the middle part, owing to the presence of a large, elongated ovary. The vulva is situated in the posterior third of the animal, and the uterus contains usually five or six rather elongated ova. The anus is situated toward the base of the tail. The eggs are of a yellowish-green color, rather opaque, and apparently finely granular (Bavay); in their general appearance they resemble those of the uncinaria (Fig. 116).

Fig. 115.—Larvæ of strongyloides intestinalis. × 100. (From Bull. 1, 1913, Surgeon-General's Office.)

While infection originally takes place through the filariform larvæ of Anguillula stercoralis, an auto-infection may also occur without the intervention of the sexually differentiated forms, by a direct transformation of the rhabditiform embryos of the parasitic mother animal, and there is evidence to show that this latter cycle is indeed the more common. There is no evidence to show that the sexually mature intermediate generation ever develops in the intestinal tract during life. The time elapsing between infection with the filariform larvæ and the appearance of rhabditiform embryos in the stools is about seventeen days.

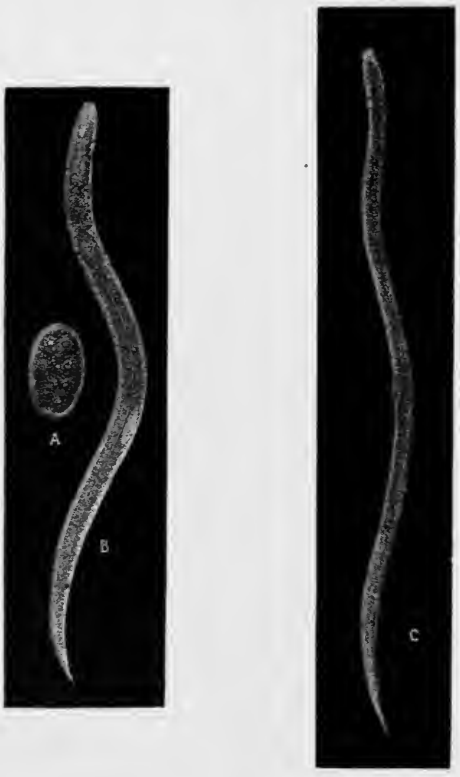


FIG. 116.—*A*, egg of Strongyloides intestinalis (parasitic mother worm); *B*, rhabditiform embryo; *C*, filariform embryo, derived by direct transformation from a rhabditiform embryo. (Taken from Thayer.)

The accompanying diagram illustrates in brief this apparently complicated cycle of development (taken from Brumpt):

INTESTINAL FORM
Asexual; gives rise by parthenogenesis to
↓
OVA
Which at times in the intestine of man, but more frequently outside of the body, give rise to a
↓
FIRST GENERATION OF RHABDITIFORM LARVÆ
Outside of the body at a temperature of 15° to 18° C., after moulting, these are transformed into strongyloid larvæ. At a higher temperature (20° to 25° C.) they become differentiated into sexual forms:
MALE AND FEMALE TYPE OF S. STERCORALIS
After sexual union the females lay
OVA
Whence arise ↓
A SECOND GENERATION OF RHABDITIFORM LARVÆ
Which moult and then become
STRONGYLOID LARVÆ
These enter the human body through the skin or the alimentary tract and thus become the
INTESTINAL FORM

The parasite is the recognized cause of the so-called Cochin-China diarrhea, and is of further interest from its resemblance to the common hookworm, with which it is not infrequently found associated. Excepting in very rare instances, it does not cause intestinal ulceration, and it is supposed that the injurious effects of the parasite are purely mechanical. It is possible, however, that these may also be owing to the irritating action of its excretory products. The clinical manifestations of the disease are mainly those of a chronic diarrhea and a comparatively mild anemia. There are usually three or four pasty stools a day.

The organism was first discovered in individuals who had contracted severe diarrhea in Cochin-China. Grassi and Parona later found the worm in Italy, and at the building of the St. Gothard tunnel it was frequently seen in association with the hookworm. Thayer was the first to find it in the United States, and it is interesting to note that two of his three cases may have become infected in either Maryland or Virginia. The third case may have originated in Austria; in it the anguillula was associated with amebas and the Trichomonas intestinalis; it ended fatally, being complicated by liver abscess. Since then additional cases have been reported in the United States by Moore, Price, Lamar and others.

Other cases have been observed in Belgium, Holland, Martinique, Brazil, Sicily, the Dutch Indies, Egypt, Germany, Spain, and the Philippine Islands.

CHEMISTRY OF THE FECES

Reaction.—The reaction of the feces is under normal conditions usually alkaline, sometimes neutral, rarely acid, the alkalinity being due to ammoniacal fermentation, the acidity to lactic and butyric acid fermentation.

In disease also the reaction of the stools is variable and of little clinical interest. In typhoid fever an alkaline reaction is almost constantly met with; it may, however, also be neutral, amphoteric, or even acid. In acute infantile diarrhea an acid reaction is the rule, but exceptions also are not infrequent. Normal stools of sucklings are acid, the degree of acidity, according to Langstein, corresponding to about 2.1 to 3.7 per cent. of normal NaOH for 100 grams of the moist feces.

General Composition.—A general idea of the chemical composition of the feces may be formed from the following roughly classified list of its components:

1. Food material which could be assimilated, such as starches, fats, and a small mount of non-assimilated albuminous material.

2. Indigestible substances, such as chlorophyll, gums, pectic products, resins, various coloring matters, nucleins, chitin, and insoluble

salts, viz., silicates, sulphates, earthy phosphates, ammonio-magnesium phosphate, etc.

3. Products derived from the digestive canal, as mucus, partly transformed biliary acids, dyslysin, cholesterin, lecithin.

4. Substances in process of absorption, as emulsified fats, fatty acids, leucin, and biliary acids.

5. Products of decomposition, referable to microbic activity, such as fatty acids, comprising the entire series from acetic to palmitic acid, the latter being especially abundant; lactic acid, phenol, cresol, indol, skatol, excretin, leucin, and tyrosin; phenyl-propionic, phenyl-acetic, hydroparacumaric, and parahydroxy-phenyl-acetic acids; ammonium carbonate, and ammonium sulphide.

6. Products of metabolism eliminated through the intestines; urea, uric acid, and xanthin bases.

7. Pigments: stercobilin, hydrobilirubin, and, under abnormal conditions, bile pigment and blood.

8. Water.

9. Carbon dioxide, marsh gas, hydrogen, nitrogen, and, under abnormal conditions, hydrogen sulphide, methyl merceptan, etc.

For a chemical consideration of the majority of these components the reader is referred to special works on physiological chemistry; thus far they do not play a role in clinical diagnosis. Only a few are considered at this place.

Cholesterin.—Cholesterin ($C_{26}H_{44}O$) occurs in small amounts in almost all animal fluids. It is found also in various tissues of the body, especially in the brain, and, as has already been pointed out, it is the most important component of the usual variety of gallstones. Its origin and mode of formation in the various organs of the body, as well as the cause of its presence in the alimentary canal, are unknown. It crystallizes in colorless, transparent plates, the margins and angles of which usually present a ragged appearance. It is practically insoluble in water, dilute acids, and alkalies. In boiling alcohol it is readily soluble and crystallizes out from this solution on cooling; it is likewise easily soluble in ether, chloroform, and benzol.

Tests for cholesterin: 1. Under the microscope add a drop of concentrated sulphuric acid to some of the crystals; they gradually disappear, the edges assuming a yellowish-red color.

2. Dissolve a few crystals in chloroform, add concentrated sulphuric acid, and shake the mixture; the chloroform assumes a blood-red to a purplish-red color, while the sulphuric acid at the same time shows marked fluorescence.

The Biliary Acids.—The biliary acids found in the feces are glycocholic acid ($C_{26}H_{43}NO_6$), taurocholic acid ($C_{26}H_{45}NSO_7$), and cholalic acid ($C_{24}H_{40}O_5$).

The two former occur normally in the bile, and can be decomposed into cholalic acid and glycocoll, and cholalic acid and taurin respec-

tively; as this process of decomposition takes place ordinarily in the intestines, the third acid—*i. e.*, cholalic acid—is always found in the feces.

In order to demonstrate the biliary acids, the fatty acids, phenols, indol, and skatol are first removed by distillation with phosphoric acid. The residue is taken up with water and boiled, and the filtered liquid precipitated with lead acetate and a little ammonium hydrate. The biliary salts of lead are contained in the precipitate, from which they can be removed by washing with water and finally boiling the precipitate with alcohol. The washings are filtered and the lead salts transformed into sodium salts by treating the filtrates with sodium carbonate. After further filtration the filtrate is evaporated to dryness and the residue extracted with alcohol. Upon evaporation the salts of the acids sometimes crystallize out as such, while more often a dirty amorphous precipitate is obtained, which may be rendered crystalline by treating with ether. The amorphous residue, however, can be employed for making the necessary tests.

Pettenkofer's Test.—A small amount of the substance is dissolved in water and treated with two-thirds its volume of concentrated sulphuric acid, care being taken that the temperature does not exceed 60° or 70° C. While stirring a 10 per cent. solution of cane sugar is added drop by drop. If biliary acids are present the solution assumes a beautiful red color, which on standing turns a bluish violet. This test depends upon the action of furfurol, derived from the sulphuric acid and cane sugar, upon the biliary acids.

Pigments.—*Stercobilin.*—The principal pigment of normal feces is termed stercobilin, and was first isolated from this source by Vanlair and Masius. Owing to its great similarity to hydrobilirubin, it has even been regarded as identical with it, but Garrod and Hopkins have conclusively shown that whereas the urobilin of the urine and the stercobilin of the feces are identical in composition, as also in properties, they differ conspicuously from hydrobilirubin, especially in the much smaller percentage of nitrogen which they contain, viz., 4.11, as compared with 9.22 per cent. It is derived from bilirubin, and formed in the upper regions of the large intestine more especially, as the result of bacterial activity. This explains the observation that, as a rule, the meconium and the solid excreta of the first day or two of life contain no urobilin, and that the pigment also disappears, when for any reason the bile is prevented from entering the intestinal canal.

Test for stercobilin: A small amount of feces is stirred up in water and a few cubic centimeters of the resultant mixture treated with an equal amount of a saturated aqueous solution of bichloride of mercury. A normal stool, owing to the presence of stercobilin, then turns a pinkish red, which is the more marked the fresher the material. A green color is abnormal and denotes the presence of bile pigment.

Bile Pigment.—Bile pigment is normally absent from the feces. It occurs in large amounts in catarrhal conditions of the small intestine, and may be demonstrated by Gmelin's method, viz., a drop of the filtered liquid, or a particle of the colored fecal matter, is brought into contact with a drop of fuming nitric acid, when the yellow color will be seen to pass through the various shades of the spectrum, the green shade being the most characteristic. At times, however, it is not possible to obtain a positive reaction in this manner, although bile pigment is present. In such cases the examination should be conducted under the microscope, and attention directed to bile-stained epithelial cells, leukocytes, particles of mucus, and crystals.

Hematoporphyrin.—To judge from the investigations of Stokvis and Garrod, this is likewise a normal component of the feces, but occurs only in traces. Garrod states that with Saillet's method, the basis of which is extraction with acetic ether, after the addition of acetic acid, he invariably found traces, comparable with those which normally are present in the urine. He also states that he found considerably larger amounts of the pigment in the meconium, both in that expelled during the first day or two of life and in that removed from the intestines of stillborn infants.

The presence of these normal traces has been referred by some to the ingested blood-coloring matter of red meat and vegetable chlorophyll. Garrod, however, finds that the hematoporphyrin does not disappear when these articles of diet are withdrawn, and while admitting that the ingested hemoglobin and chlorophyll may possibly be converted, in part at least, into hematoporphyrin, he concludes that the greater portion is derived from endogenic sources. On the whole, the evidence seems now in favor of the view that the hematoporphyrin which is found both in the urine and in the feces originates within the liver, and is eliminated into the intestinal canal in the bile. (See also Hematoporphyrinuria.)

Purin Bodies.—The purin bases of the feces are derived from the nuclei of desquamated epithelial cells, from the nucleoproteids of bacteria and leukocytes, from the secretions of the intestinal glands and the pancreas, and from the ingested food. The normal quantity according to Schittenhelm varies between 0.1109 and 0.1669 purin nitrogen. When excessive amounts of meat, thymus gland, or guanin are added to the diet a large proportion of the purin nitrogen is eliminated in the feces in the next twenty-four hours. In diarrhea the fecal purins are increased.

Guanin, adenin, xanthin, and hypoxanthin are all represented, the first two prevailing.

Mucin.—According to Hoppe-Seyler, mucin is a constant constituent of the feces, both under physiological and pathological conditions. Normally, however, it is never possible to recognize its presence either with the naked eye or with the microscope. A satis-

factory test for the rapid demonstration of mucin in the feces does not exist. The old test of Hoppe-Seyler indicates nucleo-albumin, but not true mucin. To this end the feces are digested with water and treated with an equal volume of milk of lime; the mixture is allowed to stand for several hours, when it is filtered and the filtrate tested with acetic acid. In the presence of nucleo-albumin a cloud develops upon addition of the acid.

Albumin.—This is demonstrated in the feces by treating repeatedly with water slightly acidified with acetic acid. The filtrate is then examined for albumin according to methods given elsewhere. (See Urine.) Under normal conditions these reactions prove negative. Pathologically, serum albumin has been observed in cases of typhoid fever, in chlorosis, and in various intestinal diseases of infants.

Determination of the Residual Albumin (Koziczkowsky).—The patient is placed upon a test diet very similar to that of Schmidt and Strassburger, consisting of $1\frac{1}{2}$ liters of milk, $\frac{1}{4}$ liter of bouillon, 6 pieces of zwieback, 40 grams of oatmeal, 40 grams of butter, 2 eggs, 30 grams of finely hashed meat, and 200 grams of potato. The feces are previously demarcated by giving 0.2 gram of powdered carmine.

Two portions of stool, each representing 2 grams of dried feces,[1] are placed upon nitrogen-free filters and washed successively with ordinary ethyl alcohol (93 to 94 per cent.), absolute alcohol, and 3 per cent. hydrochloric acid. One portion (A) is then mixed with 50 c.c. of a digestive mixture of the following composition:

3 per cent. solution of hydrochloric acid	10.0
Pepsin	30.0
Water	100.0

The second portion (B) is suspended in a corresponding amount of dilute hydrochloric acid without pepsin. The total acidity and amount of free hydrochloric acid are then estimated in each by titrating with $\frac{n}{10}$ alkali solution, after which both specimens are corked and placed in the incubator over night, at 37° C. The next day the total acidity and free acid are again estimated. The difference in the amount of free acid in specimen A indicates the amount which was used in the digestion of the albumins present, and thus serves as an index of their quantity; normally this corresponds to from 15 to 18 c. c. of $\frac{1}{10}$ normal alkali. The difference in the amount of free acid in specimen B is referable to the action of proteolytic ferments (pepsin) in the feces *per se*. Normally this rarely exceeds 2 to 3 c.c. $\frac{1}{10}$ normal solution.

Albumoses.—These are normally absent from the feces. They have been observed in typhoid fever, dysentery, tubercular ulceration,

[1] One gram of formed stool represents 0.3 gram of the dried substance; 1 gram of semiliquid stool (good fat absorption) equals about 0.25 to 0 27 gram; 1 gram of semiliquid stool (with poor fat absorption) equals about 0.116 gram of dry material.

purulent peritonitis with perforation into the gut, atrophic cirrhosis, and carcinoma of the liver. Acholic stools are also usually rich in peptones.

The albumoses are demonstrated in the following manner: The feces are digested with water, so as to form a thin mush; they are then boiled, filtered while hot, and the filtrate examined for albumin, so as to be sure that all of this has been removed. The mucin is removed by treating with lead acetate, when the filtrate is examined for albumoses as described in the chapter on Gastric Contents.

Carbohydrates.—Of the carbohydrates, starch, glucose, and certain gums may be found. In order to demonstrate these the feces are boiled with water, filtered, and evaporated to a small volume. This solution may now be tested with phenylhydrazin or Trommer's reagent for glucose (see Urine), and with a solution of iodopotassic iodide for starch (see Saliva).

In normal breast-fed infants sugar is only demonstrable in traces in the stools. Langstein finds that the presence of more than traces of glucose in the stools of milk-fed infants may be regarded as a diagnostic symptom of a catarrhal process in the duodenum.

Ptomains.—Of ptomains, only two have been isolated from the feces, viz., putrescin and cadaverin. They have been found in Asiatic cholera, in cholerina, dysentery, and in connection with cystinuria. In cholera and cystinuria their amount may be quite large. Baumann and v. Udranszky obtained 0.5 gram of the benzoylated compounds from the collected feces of twenty-four hours. Such findings are exceptional, however; more often the result is negative or traces only are found; such has been my own experience and that of others. (See Ptomains in the Urine.) In cholera the cadaverin seems to predominate, while in cystinuria more putrescin is found.

To isolate the diamins in question, the feces are digested with alcohol which has been acidified with sulphuric acid. The alcoholic extract is evaporated, the residue dissolved in water, and further benzoylated, as described in the section on Urine.

Ferments.—The ferments which may be encountered in the feces are in part derived from the salivary glands, in part from the pancreas, in part from the gastro-intestinal tract, and in part from the bacterial inhabitants of the latter. Of these, the diastatic ferment of the pancreas has thus far attracted most attention and is the one that can be estimated with the greatest readiness. It was found absent by Brown in extensive carcinoma of the pancreas, markedly diminished in chronic pancreatitis, while in achylia gastrica normal values were obtained.[1]

[1] For details regarding the method of examination see Brown, Johns Hopkins Hospital Bull., July, 1914, p. 200.

Meconium.—Meconium is a thick, tenacious, greenish-brown material which has accumulated during the intra-uterine life of the infant. Microscopically a few cylindrical epithelial cells, a few fat droplets, numerous cholesterin crystals, bilirubin crystals, and lanugo hairs are found.

Microörganisms are absent, but soon after suckling has commenced they appear in abundance. The most important of those which are then constantly present are the Bacillus lactis aërogenes, which predominates in the small intestine, and the Bacillus coli communis, which is found more particularly in the large intestine. Both have already been described. In addition to these, the Proteus vulgaris, Streptococcus coli brevis, Micrococcus ovalis, tetragenococcus, Saccharomyces cerevisiæ, Saccharomyces rubra, and a few less important microörganisms have been found.

Chemically, meconium contains bilirubin in considerable amount (recognizable by Gmelin's reaction), biliary acids, fatty acids, chlorides, sulphates, phosphates of the alkalies, and their earths. It does not contain urobilin, glycogen, albumoses, lactic acid, tyrosin, or leucin.

An idea may be formed of its composition from the following analysis of Zweifel:

Water	79.8 to 80.5 per cent.
Solids	19.5 to 20.2 "
Mineral matter	0.978 "
Cholesterin	0.797 "
Fats	0.772 "

CHAPTER V

THE NASAL SECRETION

In the nasal secretion, which normally is small in amount, transparent, colorless, odorless, tenacious, and of a slightly saline taste, pavement-epithelial cells in large numbers, ciliated epithelial cells, as well as some leukocytes and an enormous number of microörganisms, are found. Its reaction is alkaline.

In acute coryza the amount is diminished at first, but soon a very copious secretion occurs, which contains numerous epithelial cells and microörganisms. When complicated with an ulcerative condition pus is observed in considerable amount.

Occasionally, as in cases of traumatism, cerebral tumors, etc., cerebrospinal fluid is discharged through the nose, and may be recognized by the fact that it is free from albumin and contains a substance which reduces Fehling's solution.

Of pathogenic organisms, the tubercle bacillus and the bacillus of glanders may occur in ulcerative diseases of the nose, their presence indicating the existence of the corresponding affection. In ozena a large diplococcus has been described by Löwenberg, which is said to be characteristic of the disease. Oidium albicans has been observed in rare cases. The Meningococcus intracellularis of Weichselbaum, which is the cause of epidemic cerebrospinal meningitis, has even been demonstrated in the nasal secretion of healthy individuals. In acute anterior poliomyelitis the infecting agent probably gains entrance to the body through the nose, but is apparently ultramicroscopic. In ordinary cases of coryza the Micrococcus catarrhalis is frequently found.

Ascarides and other entozoa have been eliminated through the nose.

Charcot-Leyden crystals have been observed in the nasal secretion in cases of bronchial asthma and in connection with nasal polypi. Their presence is usually accompanied by the simultaneous occurrence of large numbers of eosinophilic leukocytes.

Tunnicliff has recently described an anaërobic bacillus (?) which was present in all cases of acute rhinitis examined during the early stage of the coryza. The organisms generally appear in clumps and are best stained with carbol gentian violet or carbol fuchsin, but are colored only faintly even by these. They are not stained by Gram, methylene blue, thionin, or the Giemsa stain. They measure from 5 to 8μ in length and from 0.3 to 0.5 in width. They are strict anaërobes, and develop on alkaline blood-agar in from five days to a month.

CHAPTER VI

THE SPUTUM

GENERAL EXAMINATION OF THE SPUTUM

General Technique.—The sputum should be collected in receptacles so constructed as to permit of their complete and easy disinfection. The paper spit-cups which are figured in the accompanying illustrations (Figs. 117 and 118) are admirably adapted for this purpose, as they may be destroyed immediately after use.

When working with sputa which are known or suspected to be of tubercular origin, the greatest care should be exercised to keep the expectoration from drying and becoming disseminated in the air. Negligence in this respect may result in the most serious consequences.

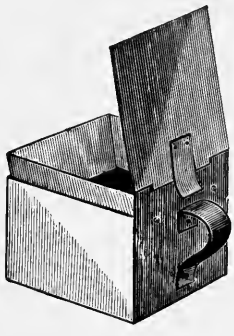


FIG. 117

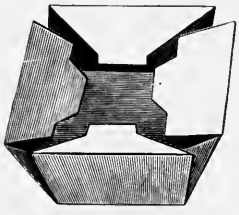


FIG. 118

Sanitary spit-cups.

The macroscopic examination of sputa is most conveniently carried out by placing small portions of the material upon a plate of ordinary window glass, of suitable size, which has been painted black upon its lower surface, and covering the same with a second, smaller plate. If it is desired to examine individual constituents which have been discovered in this manner, the upper plate is slid off until the particle in question is uncovered, when it may be removed to a microscopic slide and examined under a higher power.

It is also very convenient to have a portion of the laboratory table painted black, when unstained plates of glass may be utilized.

If these measure about 15 by 15 cm. and 10 by 10 cm. respectively, fairly large quantities of sputa may be examined *in situ* with a low power.

General Characteristics of Sputa.—Amount.—The amount of sputum expectorated in the twenty-four hours varies within wide limits, depending largely upon the nature of the disease. Thus, only a few cubic centimeters may be eliminated, or the amount may reach 600 to 1000 c.c., and even more. Very large quantities are expectorated in cases of pulmonary hemorrhage and edema of the lungs, sometimes following thoracentesis, also following perforation of accumulations of pus from the thoracic or abdominal cavities into the respiratory passages; furthermore, in cases in which large vomicæ of tubercular or gangrenous origin exist, and finally in cases of abscess of the lung, bronchiectasis, and even in simple bronchial blennorrhea. In incipient phthisis, acute bronchitis, and in the first and second stages of pneumonia, on the other hand, the amount is usually small.

Consistence.—The consistence of the sputum corresponds, in a general way at least, to its amount, and may vary from a liquid to a highly tenacious state. The cause of the tenacity of the sputum is but imperfectly understood. Mucin does not appear to be the most important factor, as this occurs in diminished amount in pneumonic sputa, which are noted for their high degree of tenacity. Kossel has suggested that the phenomenon may be due to the presence of nucleins or nuclein derivatives, while others refer it to the presence of abnormal albuminous bodies of unknown character. However this may be, sputa are not infrequently seen when it is possible to invert the cup without losing a drop of its contents. This is observed especially in cases of acute croupous pneumonia up to the time of the crisis, providing that catarrh of the bronchi does not exist at the same time. It is noted, furthermore, immediately after an attack of acute bronchial asthma, and also in the initial stage of acute bronchitis. In cases of edema of the lungs, on the other hand, the sputa are liquid and present the general characteristics of blood serum, being covered, like all albuminous liquids when brought into contact with the air, by a frothy surface layer. The sputa observed in cases of acute pulmonary gangrene, pulmonary abscess, putrid bronchitis, and following perforation into the lungs of an empyema or an accumulation of pus situated beneath the diaphragm are fluid and consist of pure pus.

Color.—The color of the sputa may vary greatly. They may be perfectly clear and transparent, gray, yellow, green, red, brown, and even black. Purely mucoid expectoration is almost transparent and colorless, as is also the sputum of pulmonary edema when not mixed with blood or pus.

The larger the number of leukocytes the more opaque does the sputum become, assuming at first a white, then a yellow, and finally

a greenish color, the latter being usually indicative of the presence of pus. The green color, however, may be due to other causes. Green sputa may thus be observed when bile pigment has become admixed to the sputa, as in cases of liver abscess perforating into the lung, or in cases of jaundice, and especially in pneumonia during lysis, in pneumonia ending in abscess, and in subacute caseous pneumonia. The same is seen in pulmonary chloroma and may also occur in pulmonary carcinoma. In cases of amebic liver abscess with perforation into the lung the sputa usually present a color resembling anchovy sauce, which is very characteristic.

The inhalation of particles of carbon gives the sputum a grayish or even a black color; the same or an ochre yellow or red color is observed in cases of siderosis due to oxide of iron. Blue sputa are seen in workers with blue dyes (methylene blue, ultramarine), etc.

A red color is usually indicative of the presence of *blood*, the shade depending upon the character of the disease. It is seen especially after the formation of cavities, in caseous pneumonia, in incipient phthisis, heart disease, etc. The shade will further depend upon the length of time that the blood, no matter what its origin may be, has remained in the lungs. In pulmonary gangrene a dirty, brownish-red color is observed, owing to the presence of methemoglobin, and, to some extent also, of hematin. Quite characteristic is the chocolate color which is observed when a croupous pneumonia terminates in necrosis and gangrene. Equally characteristic is the rusty and prune-colored expectoration seen in ordinary cases of pneumonia. Occasionally a bread-crust brown is observed in cases of gangrene and abscess of the lung, the color being due to the presence of hematoidin or bilirubin. A light brown color may be seen in cases of chronic passive congestion, as in mitral disease.

Odor.—Most sputa are odorless. Under certain conditions, however, there may be a marked odor. In cases of pulmonary gangrene or putrid bronchitis the stench is frightful. A somewhat similar, slightly sweetish odor is observed in certain cases in which putrefactive organisms have entered the lungs, and there exert their action upon the accumulated sputa, in the absence of gangrene, as in cases of bronchiectasis, perforating empyema, and where ulcerative processes are taking place in the lungs, whether these be of tubercular origin or not. An odor like that of old cheese is occasionally observed in cases of perforating empyema; under such conditions tyrosin is usually found. This body, however, has nothing to do with the odor of the sputa; both factors are merely indicative of certain putrefactive changes going on in the lungs.

Specific Gravity.—The specific gravity of sputa varies within wide limits; mucous sputa have a specific gravity of 1.004 to 1.008, purulent sputa one of 1.015 to 1.026, and serous sputa one of 1.037 or more.

Configuration of Sputa.—As a general rule, the following forms of sputa, which may be termed pure sputa, present a homogeneous appearance:

Mucoid sputa,
Purulent sputa,
Serous sputa,
Sanguineous sputa,
} Homogeneous sputa,

with one exception, perhaps—the typically rusty sputa of croupous pneumonia; while mixtures of any two or three of these may be classed as heterogeneous sputa:

Mucopurulent sputa,
Mucoserous sputa,
Serosanguineous sputa,
Sanguino-mucopurulent sputa,
} Heterogeneous sputa.

The so-called *sputum crudum* of the first stage of acute bronchitis may be regarded as an example of a purely mucoid sputum. A purely purulent sputum is usually indicative of the perforation of an empyema or any other accumulation of pus into the lungs or bronchi, of pulmonary abscess, or of bronchial blennorrhea. A purely serous sputum is found in cases of pulmonary edema, and a purely hemorrhagic sputum in cases of pulmonary hemorrhage.

Of the heterogeneous sputa, the most important are the so-called *nummular sputa* of the second and third stages of phthisis. These are characterized by the fact that when thrown or expectorated into water they sink to the bottom, and there form coin-like disks, from which property they have received their name. Such sputa are mucopurulent in character, and contain a focus of almost pure pus embedded in a more or less homogeneous mass of mucus. Quite different from these are the so-called *sputa globosa*, which consist of fairly dense, roundish, grayish-white masses; they are secreted in old cavities which have become lined with a granulation membrane.

Occasionally, as in putrid bronchitis, bronchorrhea, bronchiectasis, and gangrene of the lungs, exquisite *sedimentation* is observed. Such sputa when collected in a conical glass present three distinct zones: The one at the bottom contains the cellular elements; the second the pus serum; the third or superficial layer consists of mucus and contains many air bubbles. From this, long shreds of sedimentous material sometimes hang down.

Macroscopic Constituents of Sputa.—Cheesy Particles.—The presence of small, *cheesy particles*, which are occasionally found at the bottom of the spit-cup, is sometimes very important. They vary in size from that of a millet-seed to that of a pea, and are observed especially in the second and third stages of phthisis. Usually they contain tubercle bacilli in large numbers, and frequently also elastic tissue.

Not to be confounded with these are small, caseous masses which are at times expectorated by perfectly normal individuals, and also by patients suffering from disease of the tonsils, ozena, etc., and which in part come from the tonsils or mucous cysts (Dittrich's plugs); others may be derived from the bronchi. Formerly they were regarded as tubercles, and in apprehensive individuals their expectoration may cause a great deal of anxiety. As a rule, they are expectorated unaccompanied by pus or even mucus; rubbed between the fingers they emit an extremely offensive odor, which is referable to the presence of fatty acids; microscopically they consist of bacteria, fatty acid crystals, fat globules, and cellular detritus.

Elastic Tissue.—In cases in which active parenchymatous destruction of the lungs is going on bits of elastic tissue may be found which are visible to the naked eye. The search is facilitated by spreading out the sputum between two plates of glass, upon a dark background, and searching with a hand lens. In tuberculosis the particles are quite small, while in abscess and gangrene they may attain the size of a pea. Their macroscopic demonstration should be followed by a careful microscopic examination (which see).

Particles of cartilage from tubercular ulcers of the larynx, trachea, and bronchi are less common, as is also the occurrence of tumor fragments.

Fibrinous Casts.—Fibrinous casts are observed in croupous pneumonia, immediately before or after resolution has taken place, as also in fibrinous bronchitis (Fig. 119), and in diphtheria when the membrane has extended into the finer ramifications of the bronchi. These casts may vary in size from 15 cm. in length by several millimeters in thickness to fragments which measure only from 0.5 to 3 cm. in length. The casts observed in pneumonia, usually from the third to the seventh day, are of the latter size or even smaller, being derived from the ultimate twigs of the finest bronchioles. Those found in fibrinous bronchitis stand between these two in size, being casts of smaller and medium-sized bronchi. Attention is usually attracted to the presence of such casts by their white color; often, however, they are yellowish brown or reddish yellow, owing to the presence of blood-coloring matter; at other times they are enveloped in mucus, when their recognition may become quite difficult. Such casts are fairly firm; they branch dichotomously, usually six to ten times. The larger branches contain a lumen, while the smallest twigs are solid. Microscopically they consist of a large number of fibers, which are arranged longitudinally or in a net-like manner, and contain blood corpuscles and epithelial cells in their meshes. When treated with Weigert's fibrin stain they are sometimes beautifully resolved; at other times the fibrin reaction is not nearly so marked as one would expect. The individual casts consist of a variable number of lamina arranged concentrically, those contained in the centre being

much folded and involuted. Most of the branches are cylindrical; some of the larger ones are flat. Charcot-Leyden crystals have at times been observed in these formations.

Small casts composed of the mycelium of fungi have also been described.

Whenever it is desired to examine sputa for casts it is best to pick out particles that look promising, upon a dark surface, and then to shake them out in water.

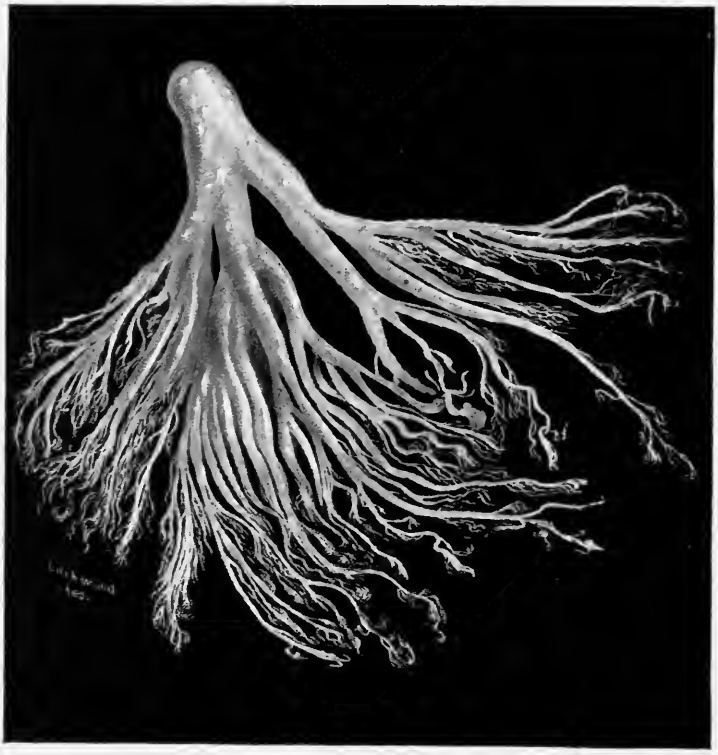


Fig. 119.—Expectorated cast from a case of fibrinous bronchitis. Three-fourths natural size. Drawn from fresh specimen. (After Bettmann.)

Curschmann's Spirals.—Quite distinct from the formations just described are the so-called spirals of Curschmann, which are observed especially in cases of true bronchial asthma, but occur also in acute and chronic bronchitis, in croupous pneumonia, and in chronic phthisis, though to a far less extent. Upon careful examination they will be seen to consist of thick, yellowish-white masses, which exhibit a spirally twisted appearance, and are characterized, moreover, by their more solid consistence and light color. Microscopically they are composed of a spirally twisted network of extremely delicate

fibrils containing epithelial cells and numerous leukocytes; the latter are almost all of the eosinophilic variety. Usually, but not invariably, Charcot-Leyden crystals also are seen. The spirally twisted mass is found to be wound around a central very light and clear thread, which usually has a zigzag course (Fig. 120).

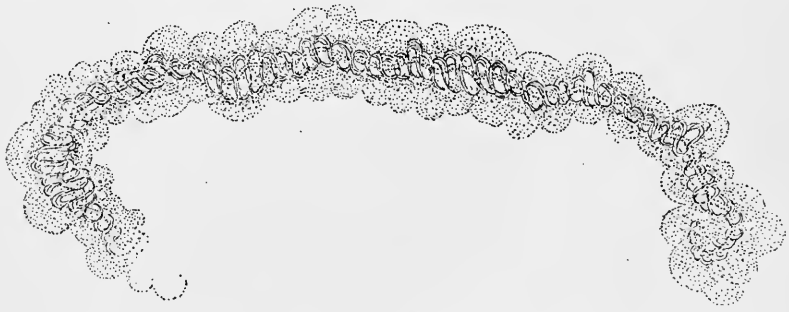


FIG. 120.—A Curschmann spiral from a case of true bronchial asthma (enlarged).

Other formations, probably mere varieties of those just described have also been observed, in which the central thread is absent or in which the spiral arrangement is deficient. The spiral form, however, with the central thread, must be considered as the most characteristic. Their length and breadth may vary a great deal, but rarely exceed 1 to 1.5 cm. Their occurrence seems always to indicate a desquamative catarrh of the bronchi and alveoli, but practically

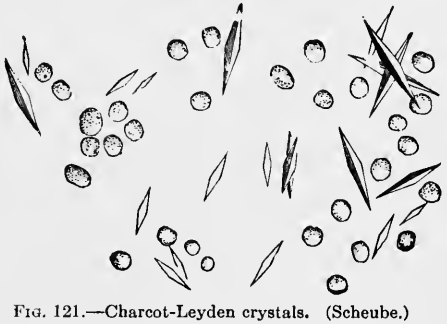


FIG. 121.—Charcot-Leyden crystals. (Scheube.)

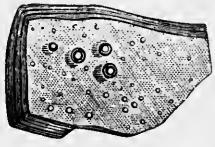


FIG. 122.—Wall of a hydatid cyst, showing the laminated structure; not magnified. (Davaine.)

nothing is known concerning their formation. If in a given case the diagnosis rests between true bronchial and what may be termed reflex asthma, the presence of these formations points to the existence of the former disease. Chemically, the spirally wound mass seems to consist of a mucinous substance, while the central thread is possibly of fibrinous origin.

Charcot-Leyden crystals (Fig. 121), which are usually absent at

the beginning of an attack of asthma, at which time only the spirals are observed, may develop in the spirals when these are kept for several days. They will be considered later in studying the chemistry of the sputum.

Echinococcus Membranes.—Echinococcus membranes may come from a perforating cyst of the liver, kidney, or lung. They constitute rather thick, and at the same time tough, pieces of membrane (Fig. 122); occasionally entire sacs are seen, of the color of white porcelain, in sections of which it is possible to make out a fibrillated structure. (See also Animal Parasites in the Sputum.)

Concretions.—The expectoration of concretions which have been formed in dilated portions of the bronchi or in tubercular cavities, or of calcified bronchial glands that have found their way into the lungs, is rare. Curious examples of the occurrence of such concretions have been reported. Andral cites a case of phthisis in which, within eight months, 200 stones were expectorated, and Portal mentions a case in which 500 were thus expelled.

Foreign Bodies.—Foreign bodies which have accidentally entered the air passages and have remained there for a long time may also be found in the sputum. Heyfelder mentions a case in which a man coughed up a wooden cigar-holder with pus and blood after eleven and one-half years. In certain cases of hysteria, material may be shown the physician as having been expectorated which has been purposely placed in the sputum or in the mouth, owing to a craving to excite special interest. In a case of this kind I found chicken lung, which the patient claimed to have expectorated.

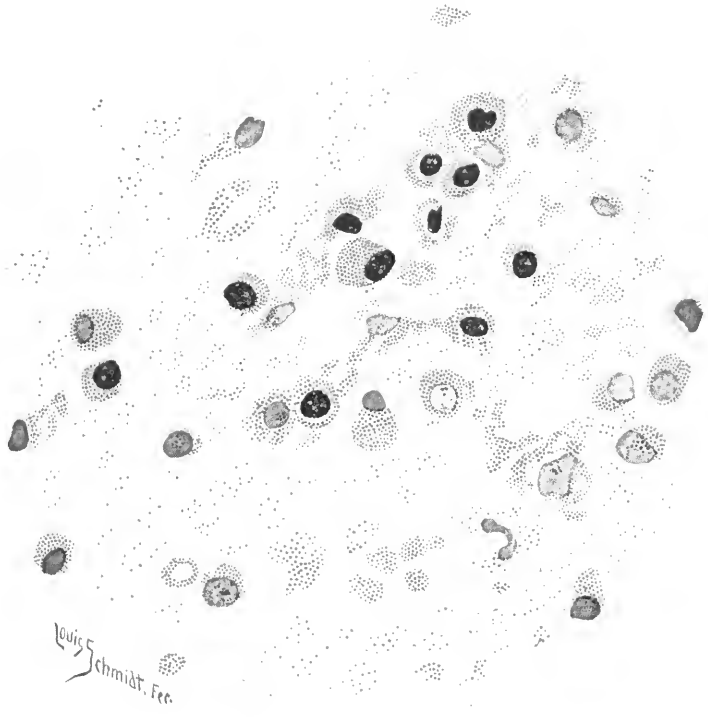
MICROSCOPIC EXAMINATION OF THE SPUTUM

Under this heading it is necessary to consider leukocytes, red blood corpuscles, epithelial cells, elastic fibers, corpora amylacea, parasites, and crystals.

Leukocytes.—Leukocytes, usually polynuclear in character, are found in every sputum, in considerable numbers, embedded in a homogeneous, more or less tenacious material. At times they contain fat droplets, or granules of pigment, such as carbon or hematoidin. Their number varies considerably, being naturally greatest in cases of perforating abscess, empyema, putrid bronchitis, etc.

While the leukocytes which usually are found in the sputum are of the neutrophilic variety, eosinophiles may also be observed, especially in asthmatic sputa, in which they predominate. Free eosinophilic granules are then also seen, and I have repeatedly observed specimens in which the spirals (see above) were literally covered with these granules (Plate XX). The presence of eosinophilic leukocytes is, however, not characteristic of the sputa of

PLATE XX



Sputum from Case of Bronchial Asthma, showing Large Numbers of Eosinophilic Leukocytes and Free Granules.

It will be noted that the leukocytes are all mononuclear. (Eye-piece 1, objective 1-8, Bausch & Lomb.)

bronchial asthma, as they may be met with in other diseases as well. Teichmüller has pointed out that they are present in a large percentage of tubercular cases, and may be found months before tubercle bacilli can be demonstrated. He regards their occurrence as evidence of a defensive struggle on the part of the body, and attaches prognostic value to their presence and number. Ott, Fuchs, Bettmann, Turban, and Cohn, on the other hand, deny the prognostic significance of the eosinophilic cells in cases of phthisis, and Cohn states, as the result of an examination of 100 cases, many of which were comparatively early, that the occurrence of eosinophilic leukocytes is fairly uncommon in tubercular sputa. Stadelmann also states that he has been unable to verify Teichmüller's observations. On the other hand, he has been able to confirm the observation which has been repeatedly made, that large numbers of eosinophilic cells appear in the sputum following hemoptysis. Teichmüller has also described an "eosinophilic" bronchitis, which is said to differ from other forms of the disease in the abundance of eosinophilic cells which are encountered. The sputum in such cases is described as transparent, mucoid, and loose, with yellow, purulent admixtures. It is said to be markedly different from the tough, thick sputa of bronchial asthma. Typical spirals are absent, but rudimentary forms may be encountered. Charcot-Leyden crystals are present. I have myself seen a few instances of this kind (without crystals) in which asthmatic symptoms did not exist.

Very curiously, the majority of the eosinophilic cells which are met with in the sputum (notably in asthma) are mononuclear; they are not myelocytes, however, but probably mononuclear histogenetic forms.

To demonstrate eosinophilic leukocytes in the sputum, smears are made as usual, slightly fixed by drawing through the flame of a burner, and stained for two minutes in a 0.5 per cent. alcoholic solution of eosin. The preparations are then immersed in 50 per cent. alcohol to the point of decolorization, when they are counterstained with methylene blue, briefly washed with water, and dried. The eosinophilic granules and the red cells in part hold the eosin dye.

Basophilic leukocytes (mast-cells) have also been observed in the sputa.

Red Blood Corpuscles.—The presence of red blood corpuscles in small numbers does not by any means indicate serious pulmonary or cardiac disease, as they may be found in almost any sputum, and especially in that of individuals who smoke much or live in a smoky atmosphere; they are, without doubt, derived from the catarrhally inflamed bronchial or tracheal mucosa. Whenever they occur in large numbers, however, their presence becomes important. They may be observed in acute bronchitis, pneumonia, edema of the lungs, bronchiectasis, abscess, gangrene—in fact, in all pulmonary

diseases. Their occurrence is most important in phthisis, and is, in fact, one of the most constant symptoms of the disease.

The form of the red corpuscles will depend upon the length of time that they have remained in the lungs, and all gradations from the typical red corpuscle to its shadow, or even fragments, may be observed. In pneumonia the microscopic examination may at times be disappointing, the appearance of the sputum suggesting that red corpuscles in large numbers are present, while, as a matter of fact, they are almost all destroyed, the color being due to altered pigment. It may even be necessary to depend upon chemical methods to clear up the question. It should be remembered that the presence of blood pigment is not always indicated by a red color, but that it may also assume a golden-yellow or even a greenish tinge, owing to certain chemical changes which have taken place. The golden-yellow and the grass-green sputa observed in cases of pneumonia during convalescence belong to this class.

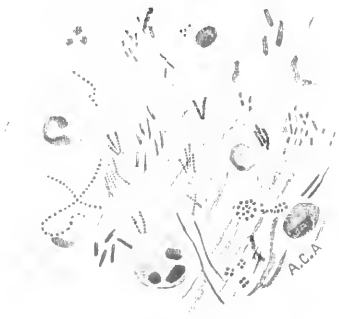
To demonstrate the presence of traces of blood in the sputum, the aloin or guaiac test (see Feces) may be employed, after first boiling the sputum with 20 per cent. caustic alkali solution and subsequently neutralizing with acetic acid.

Epithelial Cells.—Epithelial cells are found in practically every sputum. They are mostly of the pavement variety, and may be derived from the mouth, pharynx, and the upper larynx. Many of the cells are full of invading bacteria, which may lead to their entire destruction. Cylindrical epithelial cells, providing they do not come from the nose, indicate in a general way an inflammatory condition of the lower larynx, trachea, or bronchi. As a rule, their form is so much altered that it is often difficult to recognize them; they may thus become polyhedral, cuboidal, or even round, and can then hardly be distinguished from leukocytes. Actively moving cilia may be found only in perfectly fresh sputa, immediately after being expectorated, but are very rarely seen.

Formerly much importance was attached to the so-called *alveolar epithelial cells* (Fig. 123) as an aid in diagnosis. Buhl thus regarded them, particularly when undergoing fatty or myelin degeneration, to be pathognomonic of pulmonary disease, and especially of that form of pneumonia which has been termed essential idiopathic desquamative pneumonia. Bizzozero, however, as well as others, have shown that these cells not only occur in almost every known pulmonary disease, but that they are present also in the so-called "normal" expectoration which at times is obtained upon making a forcible expiration. They are round, oval, or polygonal cells varying in size from 20μ to 50μ. They may contain one, two, or three oval nuclei, which are rather small and provided with nucleoli. Usually the latter are hidden beneath numerous granules. Some of the granules are albuminous, but most of them are either pigment

PLATE XXI

FIG. 1



Tubercular Sputum Stained by Gabbett's Method. The Tubercle Bacilli are seen as Red Rods, all else is Stained Blue. (Abbott.)

FIG. 2

Heart Disease Cells, viz., Alveolar Epithelial Cells, Loaded Down with Granules of Hematin.

granules, fatty granules, or myelin granules. The *myelin granules* were first discovered by Virchow, and termed myelin granules on account of their resemblance to mashed nerve matter. They are distinguished from the other forms by their clear, pale, colorless appearance, and the fact that at times fine concentric striations can be detected. These forms may be round, but more often they are irregular. Chemically, the myelin droplets have been shown to contain a considerable amount of protagon, besides traces of lecithin and cholesterin. They are readily soluble in alcohol, somewhat so in chloroform and ether. They swell in water and stain yellow with iodine. They are colored but little by the anilin dyes and do not turn black on treating with osmic acid.

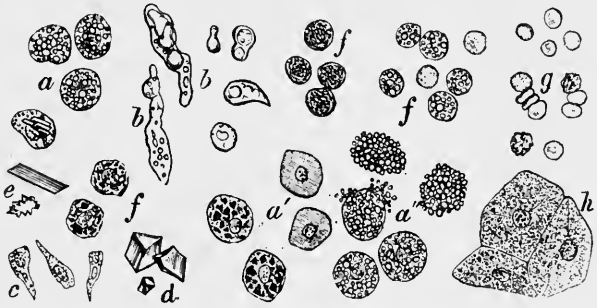


FIG. 123.—Epithelium, leukocytes, and crystals of the sputum. (Eye-piece III, objective 8 A, Reichert.) *a*, *a'*, *a''*, alveolar epithelium; *b*, myelin forms; *c*, ciliated epithelium; *d*, crystals of calcium carbonate; *e*, hematoidin crystals and masses; *f*, *f*, *f*, white blood corpuscles; *g*, red blood corpuscles; *h*, squamous epithelium. (v. Jaksch.)

Sometimes myelin granules are found, together with fatty and pigment granules in the same cell.

The sputa of chronic bronchitis referable to heart disease are characterized by the presence of so-called *heart-disease cells*. These are alveolar epithelial cells containing hematoidin granules (Plate XXI, Fig. 2). They appear to be most numerous in cases of mitral disease, but may also occur in congestive affections of the bronchopulmonary apparatus, even with the heart intact.

Liver cells may at times be observed in the sputa in cases of liver abscess, and are easily recognized by their characteristic form.

Elastic Tissue.—Much more important from a clinical standpoint are the elastic fibers and shreds of elastic tissue which may be found in sputa. They vary much in length and breadth, and are provided with a double undulating contour; they are usually curled at their ends. Very often they exhibit an alveolar arrangement (Fig. 124), which at once determines their origin.

Whenever present, elastic tissue is an absolute indication that a destructive process is going on in the lungs. It is found in cases of

abscess of the lungs, bronchiectasis, occasionally in pneumonia, pulmonary gangrene and infarct, and, most important of all, in phthisis, in which it is said to be present in 90 per cent. of all cases. This percentage, which was obtained by Dettweiler and Setzer in 1878, is unquestionably too high in comparison with what is seen today, where the diagnosis of tuberculosis is made much earlier. In gangrene of the lung elastic tissue is generally said to be absent, but Osler states that he has never seen a case without it, and that usually it occurs in large fragments.

In every case it is necessary to determine whether the elastic tissue has not been introduced from without, and it may hence be stated as a rule that it can only be regarded as absolutely characteristic when showing the alveolar arrangement.

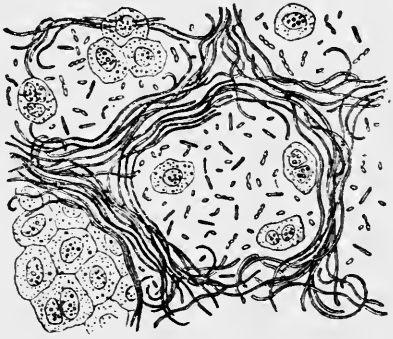


Fig 124.—Elastic fibres in the sputum. (Eye-piece III, objective 8 A, Reichert.) (v. Jaksch.)

In order to demonstrate the presence of elastic tissue in the sputum the following method is very convenient: A small amount of the thick purulent portion of the sputum is pressed into a thin layer between two pieces of plain window glass, 15 by 15 cm. and 10 by 10 cm. The particles of elastic tissue appear on a black background as grayish-yellow spots, and can be examined *in situ* under a low power. Or, the upper piece of glass is slid off until the piece of tissue is uncovered, when it is picked out and examined on a slide, first with a low and then with a higher power. At first there will be some difficulty in distinguishing with the naked eye between elastic fibers and particles of bread, or milk globules, or collections of epithelium and debris, but with practice such mistakes are rarely made, and the microscope always reveals the difference.

If only very little elastic tissue is present, it is necessary to examine large quantities of sputum with a moderately low power, and best after the addition of a solution of sodium hydrate. The sputum is boiled with a 10 per cent. solution of the reagent, an equal volume

being added; the boiling is continued until a homogeneous solution has been obtained; after dilution with four times its volume of water it is allowed to settle for twenty-four hours or centrifugalized and the sediment examined at once.

May recommends the following method of demonstrating the presence of elastic tissue in sputum: The material in question is heated on a boiling water bath with an equal volume of a 10 per cent. solution of sodium hydrate until it has all apparently dissolved. The mixture is then centrifugalized and the supernatant fluid decanted. The sediment is treated with about 2 c.c. of an orcein solution prepared according to the formula of Unna-Tänzer, viz., orcein, 1 gram; absolute alcohol, 80 c.c.; distilled water, 40 c.c.; concentrated hydrochloric acid, 40 drops. On adding the stain, owing to the remaining alkali, the color turns violet; a few drops (3 to 5) of hydrochloric acid are added until the original color of the stain returns. The tube is then placed for from two to five minutes in boiling water, after which acid alcohol (concentrated hydrochloric acid, 5 c.c.; 95 per cent. alcohol, 1000 c.c.; distilled water, 250 c.c.) is added to decolorize. The mixture is again centrifugalized and the sediment washed once or twice more with the acid alcohol by centrifugation and decantation. The sediment is then examined directly, when the elastic-tissue fibers may be recognized by their more or less intense brownish-violet color.

ANIMAL PARASITOLOGY OF THE SPUTUM

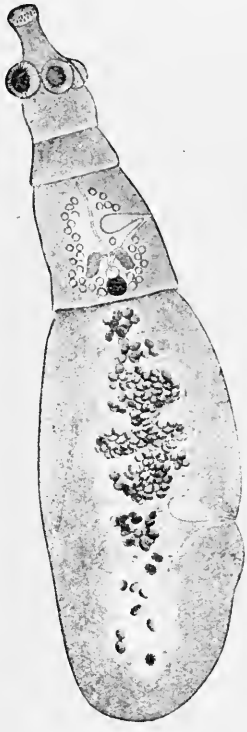
Protozoa.—Entamœba Dysenteriæ.—In cases of amebic abscess of the liver with perforation into the lung the corresponding organism may be demonstrated in the sputa. Such sputum commonly presents the anchovy-sauce appearance already mentioned. As a rule, the amebas are not numerous, and slide after slide may have to be examined before a single organism is discovered. The material should be kept at body temperature and the slides warmed. A Bausch & Lomb $\frac{1}{6}$ or Leitz 6 or 7 is used. (See also Amœbæ in Feces.) Only actively moving organisms are diagnostic.

Trichomonads have at times been observed in cases of gangrene of the lung, and in the pus removed post mortem from lung cavities. They are identical with the Trichomonas vaginalis of Donné.

Cercomonads have been found in the sputum and in the Dittrich plugs in gangrene of the lung.

Cestodes.—Tænia Echinococcus.—Portions of echinococcus cysts, viz., pieces of membrane (Fig. 122) and hooklets (Fig. 128), are occasionally seen when the parasite has lodged in the lungs or in the neighboring organs. The disease is not common in this country. Lyon collected 241 cases in the United States and Canada up to

July 1, 1901; 91 per cent. occurred in foreigners. In Canada a large proportion is referable to the Icelandic immigrants in Manitoba. Thomas, of Adelaide, has thoroughly investigated the disease in Australia, where it is quite common.



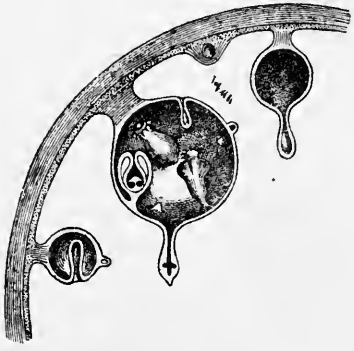


Fig. 126

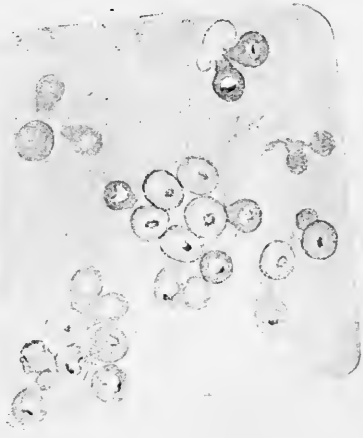


Fig. 125

Fig. 127

Fig. 125.—Tænia echinococcus. × 50. The cirrus pouch, the vagina, uterus, ovary, shell-gland and vitellogene gland, and the testicular vesicles at the sides are recognizable in the second proglottis; the uterus partly filled with eggs, as well as the cirrus pouch and the vagina.

Fig. 126.—Section through an echinococcus cyst with brood capsules.

Fig. 127.—A piece of the wall of an Echinococcus veterinorum stretched out and seen from the internal surface. × 50. A few brood capsules with scolices directed toward the interior and exterior. (Thomas.)

The adult parasite (Fig. 125), Tænia echinococcus (v. Siebold), is a three- or four-segmented tapeworm, 4 to 5 mm. in length, whose habitat is the intestinal canal of the dog, dingo, jackal, wolf, etc. The larval or cystic form develops in cattle, sheep, swine, rabbits, etc., and is also found in man. The ova, 0.067 mm. in diameter,

are introduced by food, water, or by inhalation in dust. In the digestive tract the minute embryo, freed of its resistant envelope by the digestive juice of the stomach, bores its way through the intestinal wall, and finds a resting place in the liver, lung, or other part of the body, there developing into the cystic form that may attain enormous size.

The primary or mother cyst may produce daughter cysts, these latter granddaughter cysts, and these a third generation, often in great number; so that the cavity may be filled with cysts of varying size, formed by exogenous or endogenous growth. On the other hand, the single cyst may remain sterile—acephalocyst—or may produce scolices (Fig. 126) which are attached by pedicles to the lining of the vesicles or brood capsules in which they develop. Each scolex, or echinococcus head, 0.4 to 0.25 mm. in diameter, is a round or oval body with a head capable of protrusion or retraction. There is a single or double circlet of hooklets around, and four suckers behind the rostellum. The body is partly covered with calcareous particles. These scolices may ordinarily be found in hydatid-cyst contents.

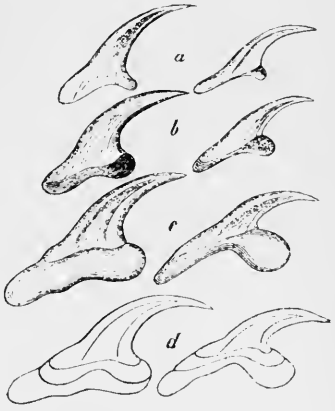


FIG. 128.—Hooklets of echinococcus: *a*, Echinococcus veterinorum; *b*, Tænia echinococcus three weeks after infection; *c*, adult Tænia echinococcus; *d*, three forms of hooklets outlined one within the other. (Leuckart.)

Hydatid membrane (Fig. 122) varies in thickness according to the size of the cyst, a mother-cyst membrane being often $\frac{1}{8}$ inch or thicker; the smaller cysts have walls of greater delicacy. It is usually pearly or grayish white, opaque, and of gelatinous consistency, but the thin walls of the daughter cysts may be perfectly clear and transparent. The membrane consists of two layers: (1) The ectocyst, of regular laminæ of chitinous-like material, readily torn on manipulation, the innermost layers whiter and softer than the outer; (2) the delicate, soft, granular endocyst, consisting of a mass of delicate polygonal cells without distinct nuclei. From this the scolices and daughter cysts are developed. The ectocyst usually lies in close apposition to the fibrous adventitious capsule formed by the organ in which the hydatid is present. "The ectocyst, known also as the cubicula by Continental writers, presents under the microscope a peculiar stratified structure which is quite characteristic. It shows no appearance of fibers or cells, and even under high magnifying powers it exhibits a nearly hyaline or at most a faintly granular appearance." (Thomas.)

When a hydatid cyst of the lung, liver, or neighboring tissue has ruptured into the larger or smaller divisions of the bronchi, quantities of clear, watery fluid, giving the characteristic tests for hydatid fluid (see Cystic Contents) may be coughed up and be found to contain perhaps:

(*a*) Small cysts full of clear fluid, from the size of a pin's head upward—the daughter or granddaughter cysts.

(*b*) Whitish, dot-like bodies just visible to the naked eye when single, or more evident when grouped together in colonies—the scolices, or echinococcus heads (Fig. 129).

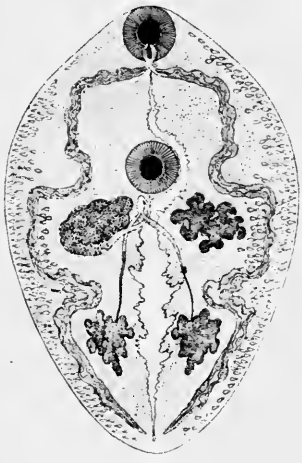


Fig. 129

Fig. 130

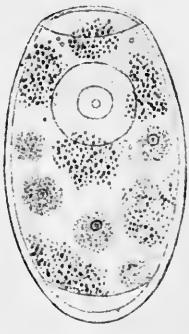


Fig. 131

Fig. 129.—Paragonimus westermanni (Kerb.). × 10. (Leuckart.) Mouth, pharynx, intestinal branches; at the sides of which the vitelline sacs are observed. The genital pore is behind the ventral sucker, and next to it, at the left, the ovary; at the right, the uterus; the two testes at the back; the excretory vessel in the middle.

Fig. 130.—Paragonimus westermanni (Kerb.) (natural size). To the left, dorsal aspect; to the right ventral aspect. (Katsurada.)

Fig. 131.—Egg of Paragonimus westermanni (Kerb.) from the sputum. × 1000. (Katsurada.)

(*c*) Some of the component parts of the cysts or scolices, viz.:

1. Collapsed cysts—the well-known "grape skins," or pieces of the gelatinous membrane of a mother or daughter cyst.

2. Hooklets and calcareous corpuscles from the bodies of the scolices visible only under the microscope.

Where the hydatid has suppurated before rupture, pus in large or small amount takes the place of the clear fluid or is mixed with it, the other elements being recognized on examination.

Microscopic Examination of Hydatid Material.—A piece of membrane (often yellowish and shreddy in degenerating cases) is picked

up with forceps, placed on a slide, a drop or two of water applied, and lightly crushed under the cover-glass. At the torn edges of the membrane the characteristic laminated structure can be readily seen with the low power (Fig. 122). It does not stain readily, but staining is unnecessary. A section may be cut with the freezing microtome and stained with carmine.

Sputa may continue to be expectorated from a hydatid cavity of the lung for months or years, and are then usually of a purulent or mucopurulent character, perhaps blood-tinged. A thick smear on a slide may reveal, when examined with a low power, pieces of laminated membrane or hooklets. A piece of membrane, if seen on floating the sputa in water, should be picked out with forceps. Tubercle bacilli are sometimes found in the sputa of cases of pulmonary hydatid. When a hydatid of the liver has ruptured into a bronchus the sputa may be bile-stained.[1]

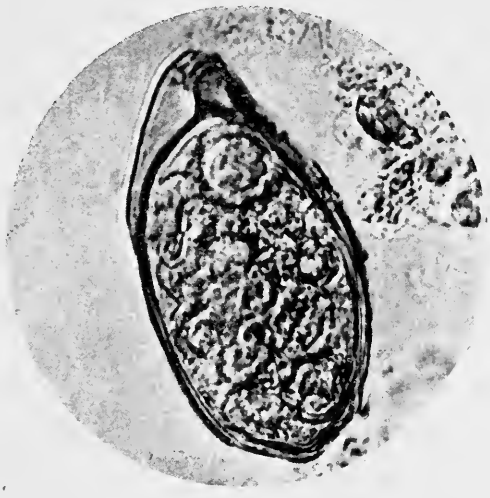


FIG. 132.—Ovum of Paragonimus westermani. × 540. (From Bull. 1, 1913, Surgeon-General's Office.)

Trematodes.—Paragonimus Westermanni (Lung Fluke).—A form of pulmonary disease closely simulating phthisis and associated with pulmonary hemorrhage is very common in Japan, and has been shown to be referable to the presence of a parasite in the lungs, Paragonimus westermanni (Kerbert): *syn.*, Distoma westermanni (Kerb.); Dist. Ringeri (Cobbold); Dist. pulmonale (Bälz). The parasite is 8 to 10 mm. long, 4 to 6 mm. wide, rounded very markedly in front, less so posteriorly. The color during life is a reddish brown. The two sucking disks are nearly equal in size. The ova are brown, with a

[1] For the above account of the component parts of hydatid material I am indebted to my friend, Dr. John Ramsay, of Launceston, Tasmania.

thin shell and lidded. They measure from 80 to 100μ in length and 40 to 60μ in breadth (Plate XXII and Fig. 132). The worm and its ova are found in the sputum. If the sputum is shaken in water and the water renewed from time to time, in the course of a month or six weeks (according to the temperature) a ciliated embryo is developed in each ovum. When the ovum is mature, on placing it on a slide and exercising slight pressure on the cover-glass, the operculum will be forced back and the embryo will emerge and at once begin to swim and gyrate in the water (Manson). The life cycle of the organism is not known. Outside of Japan the parasite has been found in China, Korea, Formosa and the Philippine Islands. Isolated cases have also been observed in the United States and Mexico. Among animals the parasite has been observed—in the United States—in dogs, cats and hogs. Many Charcot-Leyden crystals may be found in the sputum at the same time.

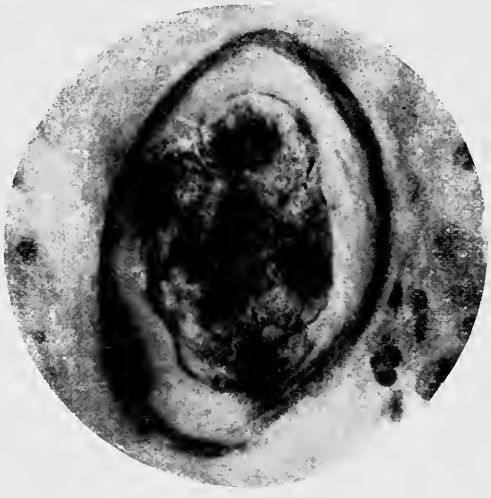


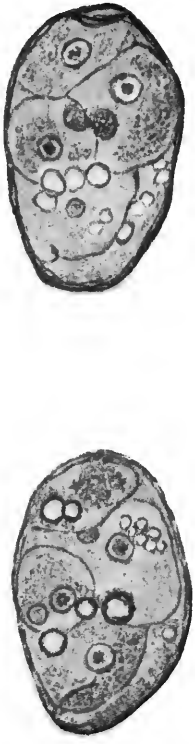
Fig. 133.—Ovum of Schistosomum japonicum. × 800. (From Bull. 1, 1913, Surgeon-General's Office.)

Schistosomum Hæmatobium.—Manson found the ova of a species of Distoma hæmatobium in the bloody expectoration of a Chinese who had lived for some time on the island of Formosa.

BACTERIOLOGY OF THE SPUTUM

The most important pathogenic bacteria which may be found in the sputa are the tubercle bacillus, the pneumococcus, the influenza bacillus, the Bacillus pertussis, the smegma bacillus, the typhoid and plague bacillus, the Micrococcus catarrhalis, Micrococcus tetragenus, staphylococci and streptococci. The general and cultural characteristics of these organisms, as well as their methods of staining, are

PLATE XXII



Ova of Paragonimus Westermanni.

described in Chapter XI. The remarks here appended have reference more particularly to their special relation to the sputum and to special technique in their demonstration.

Tubercle Bacillus.—From macroscopic examination it is impossible to decide whether or not a particular sputum is of tubercular origin. At times a sputum may have a suspicious appearance, but it is never possible to speak with certainty from simple inspection, as a mucoid sputum may contain tubercle bacilli in large numbers, while a mucopurulent sputum may be entirely free from them, and *vice versa*. Reliance should, hence, only be placed upon a careful microscopic examination.

In all cases the fine, cheesy particles previously described should be carefully sought for, as they contain the largest number of bacilli. In their absence reliance should be placed upon the examination of a large number of preparations, attention being directed especially to the purulent and mucopurulent foci of the sputum.

If but few bacilli are present the following method will be found most useful: The collected expectoration of a number of hours (or of the whole day, if need be) is treated with an equal volume or more of a 20 to 30 per cent. solution of *antiformin*. This is essentially a 10 per cent. solution of sodium hypochlorite, containing 5 to 10 per cent. of sodium hydrate. By gently agitating the mixture all the tenacious mucoid material will dissolve, and all bacteria, with the exception of the acid-fast group, are destroyed. After centrifugalization the sediment is spread on slides, and the air-dry films are fixed by heat or by immersion for several minutes in a 2 to 3 pro mille solution of bichloride of mercury, washed off, and stained as usual. (See Tubercle bacillus in bacteriological appendix.)

As the antiformin does not kill the tubercle bacilli unless exposed for a number of days, one can use the washed bacilli for purposes of culture or animal experimentation. (See also Petroff's method of culturing the tubercle bacillus from the sputum.)

After use, all glassware should be placed in a mixture of equal parts of concentrated sulphuric acid and Müller's solution for one hour or longer and then carefully washed.

In the place of antiformin one can also use Hammerl's mixture, which is a 1 per cent. solution of caustic soda in approximately a 30 per cent. solution of ammonia.

Only two bacilli are likely to be mistaken for the tubercle bacillus, viz., the bacillus of leprosy and the smegma bacillus. All three are characterized by the difficulty with which they take up basic dyes, and the great tenacity with which they hold the dye when once stained, even upon treatment with mineral acids (acid fastness) or alcohol. This peculiarity has been generally referred to the presence of fat in the bacilli, but it appears from more recent researches that the chitin or chitinous substances in the bodies of the tubercle

bacilli are primarily concerned in the reaction (Helbing). Sata, moreover, has shown that other bacteria, such as the anthrax bacillus, the bacillus of glanders, the Staphylococcus aureus, etc., give a fat reaction which is as intense as that of the tubercle bacillus, while these organisms are not in the least resistant to the action of acids when stained.

That confusion should arise in the differentiation between the tubercle bacillus and the *bacillus of leprosy* is very unlikely. More important is the *smegma bacillus*, which is known to occur at times upon the tonsils, the tongue, and in the tartar of the teeth of perfectly healthy individuals. In sputum coming from the lungs it has been observed by Pappenheim, Fränkel and others. To differentiate it from the tubercle bacillus the animal experiment or direct culture may sometimes be necessary.

For purposes of staining, Gabbett's method or the older methods of Weigert-Ehrlich or Ziehl-Neelsen are best employed (which see).

Number in Sputum.—The number of bacilli which may be found in a sputum varies greatly, and while, in general, it may be said that it is in direct ratio to the intensity of the disease, and may thus be considered of prognostic value, too much reliance should not be placed upon this statement, as in acute miliary tuberculosis, and in cases that have gone to the formation of cavities, the number may be small or they may be absent altogether. In an incipient case, on the other hand, in a little mucoid sputum the number may be large. If the number of bacilli steadily decreases in a series of examinations at intervals sufficiently long, the patient may be regarded as improving, but here the constitutional symptoms and local signs give much more accurate information.

If on repeated examination large numbers of tubercle bacilli are found, the disease has in all probability advanced to cavitation (Brown).

In tabulating the number of tubercle bacilli in reports one may adapt Gaffky's scheme, modified by L. Brown as follows ($\frac{1}{12}$ oil immersion; ocular 1; B. & L.):

1. Only 1 to 4 in a whole preparation.
2. Only 1 bacillus on an average in many fields.
3. Only 1 bacillus on an average in each field.
4. 2 to 3 bacilli on an average to each field.
5. 4 to 6 bacilli on an average to each field.
6. 7 to 12 bacilli on an average to each field.
7. 13 to 25 bacilli on an average to each field.
8. About 50 bacilli on an average to each field.
9. 100 or more bacilli on an average to each field.
10. Enormous numbers on an average to each field.

An attempt has been made to attach prognostic significance to the form and grouping of the tubercle bacilli in the sputum. To judge from the experience gathered at Saranac, it appears that virulent

and attenuated forms of tubercle bacilli possess practically the same morphology and that short bacilli usually represent a younger growth. Arrangement of the bacilli in clumps is more apt to be found in the severer cases, but may occur in all (Brown).

Diplococcus Pneumoniæ.—The pneumococcus of Fränkel and Weichselbaum is the recognized cause of acute croupous pneumonia in the majority of cases. It is then seen in the sputum in large numbers, and may be recognized by its "end-to-end" diplo-form and its capsule. It may, however, also occur in the mouth of perfectly healthy individuals, so that its diagnostic significance is somewhat limited.

The **influenza bacillus**, the **pertussis bacillus**, the **typhoid bacillus**, and the **plague bacillus** must be demonstrated by cultural methods. The **Micrococcus catarrhalis**, the **Micrococcus tetragenus**, **staphylococci** and **streptococci** can usually be distinguished by their morphological peculiarities (which see).

Streptothrices.—Within recent years there is a tendency among pathologists to abandon the older terms actinomyces, cladothrix, etc., and to speak of infection with branching mycelial organisms under the collective term streptothricosis, designating the specific variety by its special term.

Up to 1902 about 100 cases of supposed cattle *actinomycosis* had been reported in the United States as occurring in man (Ewing), but it is difficult to say how many of the older cases really belonged to this order; in the light of recent investigations it seems not unlikely that many were referable to different species.

In the cattle disease, yellow granules (so-called sulphur granules) may be found in the pus derived from actinomycotic tumors, in the sputum, and in the feces, when the disease has attacked the lungs and intestines respectively, which measure from 0.5 to 2 mm. in diameter. If such a granule is examined microscopically, slight pressure being applied to the cover-glass, it will be seen to consist of numerous threads which radiate from a centre in a fan-like manner and present club-shaped extremities (Fig. 134).

The cattle organism is termed the *Streptothrix* (*Actinomyces*) *bovis communis* (Streptothrix actinomycotica, or ray fungus). It may be demonstrated in the following manner: Dried cover-glass preparations are stained for five to ten minutes with aniline water—gentian violet (see Weigert-Ehrlich stain for tubercle bacilli), when they are rinsed in normal salt solution, dried between filter paper, and transferred for two or three minutes to a solution of iodopotassic iodide (1 to 100 or 1 to 150). They are then again dried between layers of filter paper, decolorized in xylol-aniline (1 to 2), washed in xylol, and mounted in balsam. The mycelium assumes a dark blue color. The organism is acid fast, but loses its color on washing with alcohol (95 per cent.).

In addition to the cattle cases there exists a group of pulmonary cases which present the clinical features of tuberculosis, bronchopneumonia, or gangrene, but in which the infecting agent is a species of streptothrix different from the cattle variety. About 30 cases of

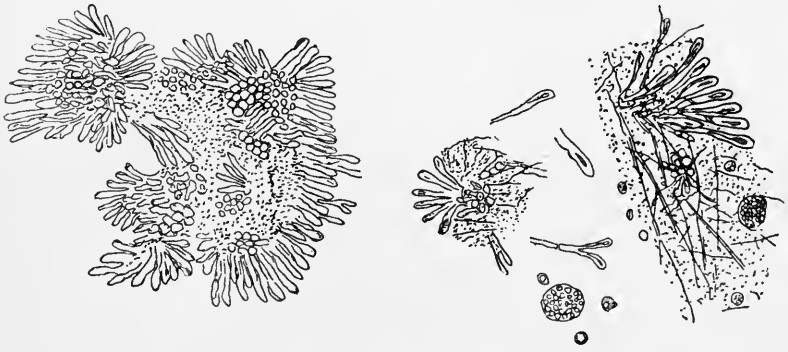


Fig. 134.—Actinomyces. (Musser.)

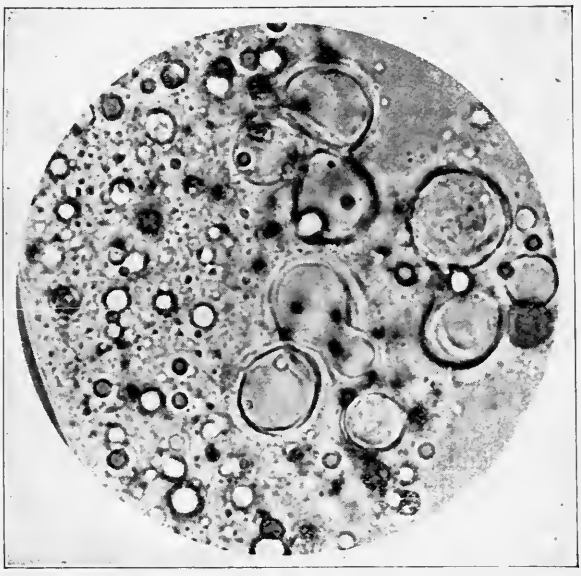


Fig. 135 —Blastomycetes. Smear from sputum mounted in 1 per cent. potassium hydrate solution showing circular and budding organisms. × 120). (Eisendrath and Ormsby.)

this kind have been reported (1906). Different species have been described, such as the Streptothrix eppingeri (Cladothrix asteroidea), Streptothrix pseudotuberculosa, Flexner; Streptothrix hominis, Foulerton; and Streptothrix israeli.

The organism is found in the sputum, often in the form of small, grayish-yellow granules. These are made up of a mycelium of branching organisms, which in the unstained specimen appear as fine, homogeneous, glistening threads, about two to four times as wide as a tubercle bacillus. They are acid fast, but can be decolorized with alcohol. In such specimens many of the threads present a beaded appearance and sometimes seem to be breaking up into short rods of varying length. With Gram some varieties stain well, while

FIG. 136.—Blastomycetes. Smear from growth on media, five weeks old, in 1 per cent. potassium hydrate solution. Low power. (Eisendrath and Ormsby.)

others do less so. Culture yields uncertain results. Flexner obtained no growth. Eppinger succeeded with gelatin, inspissated horse serum, maltose agar, and potato.

YEAST INFECTIONS

Blastomycosis.—Since the report of the first case of this kind in the United States by Eisendrath and Ormsby, some thirty cases have

been recorded in the literature, in most of which the corresponding organism could be demonstrated in the sputum. The disease is probably more common than is generally supposed and overlooked because in most sputum examinations attention is directed almost exclusively to the presence or absence of tubercle bacilli. This may be avoided, if as a matter of routine one smear at least of every case were stained with methylene blue or Jenner's, or a corresponding

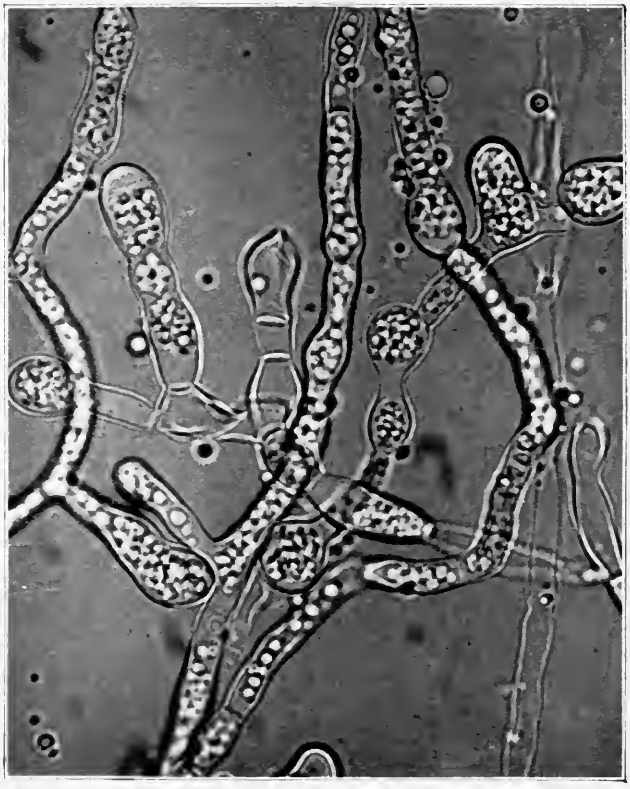


FIG. 137.—Higher magnification of Fig. 136. × 1200.

stain, when any yeast cells, whether occurring in torular (budding) or mycelial (hyphen) form would readily be seen. Or, if it is desired to examine the material unstained, one may suspend a drop of the sputum in a drop of 10 per cent. caustic alkali solution, when the structures in question are also well shown (see Figs. 135 to 137).

The organism in question is found in the sputum in torular form, its size varying from 3 to 30 micra, with an average of 20. It grows readily in the usual culture media, and irrespective of the reaction,

i. e., whether this be neutral or 0.6 per cent. acid or alkaline. Primary growth, however, takes from seven to ten days, which may later be reduced to two or three days. In litmus milk it usually produces an alkaline reaction or no change, though an initial acid reaction has

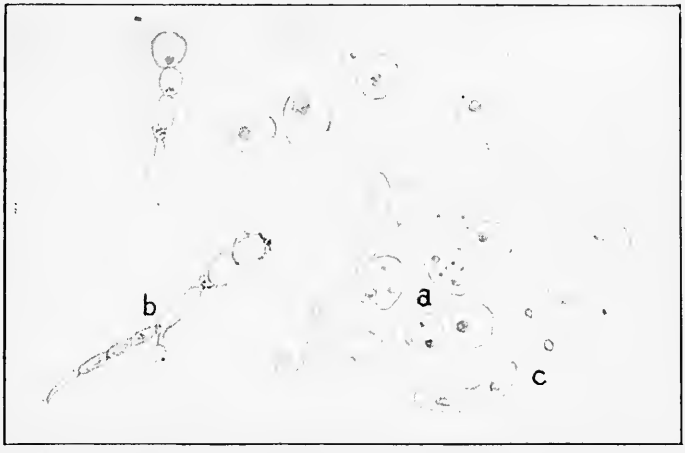


FIG. 138.—Monilia pulmonalis, showing (*a*) forms found in the sputum; *b* and *c*, premycelial forms.

been reported in a few instances; coagulation is apparently inconstant. In broth a fine sediment develops while the supernatant fluid remains clear. Dextrose, lactose, maltose and inulin are not fermented. While the organism at first multiplies by budding, mycelial formation

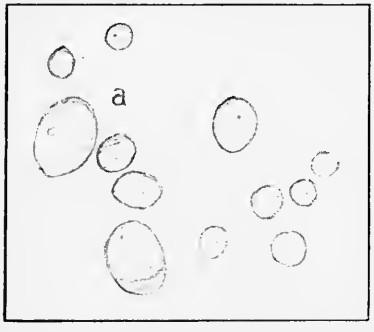


FIG. 139.—Monilia pulmonalis from young agar culture, showing vacuolated condition of the cells.

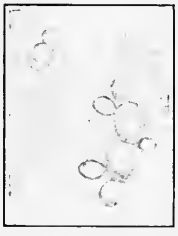


FIG. 140.—Monilia pulmonalis, from old broth culture, showing multiple budding.

occurs sooner or later with the production of aërial hyphæ. Spore formation has not been observed.

The organism is apparently non-pathogenic for the ordinary laboratory animals.

Moniliasis.—In one instance reported by Boggs and another by myself a yeast was isolated from the sputum which differed in some

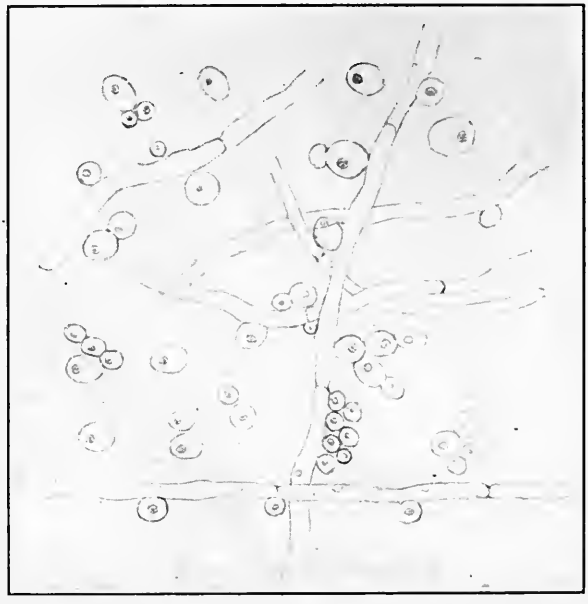


FIG. 141.—Monilia pulmonalis, showing mycelial formation and conidial production under anaërobic conditions.

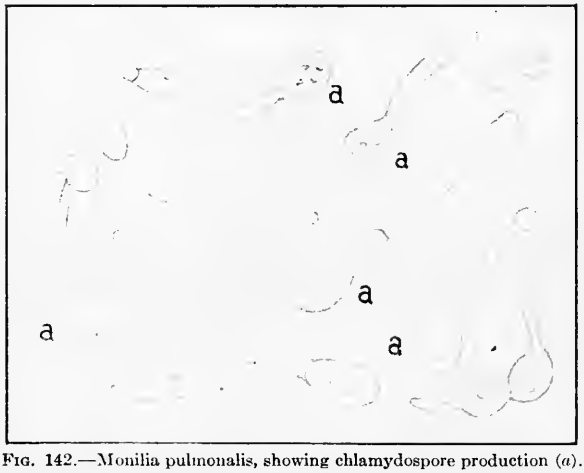


FIG. 142.—Monilia pulmonalis, showing chlamydospore production (*a*).

respects from the organism previously described in pulmonary blastomycosis. Boggs viewed his case as due to a monilia, apparently identical with that described in sprue by Bahr and Ashford. While

the organism which I found in my own case seems to be identical with that of Boggs, I feel loathe to attempt its classification, as our knowledge regarding the different types of many of the yeasts seems insufficient for this purpose. However, while that observed in the so-called blastomycosis cases hitherto reported was non-pathogenic, Boggs' organism as well as my own was markedly pathogenic. But whereas the Boggs type showed a distinct tendency to mycelial formation on prolonged cultivation, the slight tendency in that direction of my own form, diminished in the course of time, and was essentially noted under anaërobic conditions. Chlamydospore formation was noted in both, while endospores and ascospores were not observed. Primary growth on artificial media occurred within twenty-four hours. (Figs. 138 to 142.)

Coccidial Granulomatosis.—The organism which has been observed in cases of so-called coccidial granulomatosis is evidently closely related to the blastomycosis and moniliasis types, but in turn shows certain points of difference which have led to its description as a separate form. It is thus said to produce endospores and is pathogenic for rabbits and guinea-pigs. As in blastomyces there is a production of aërial hyphæ which was not observed in the monilia cases. Primary growth on artificial media may occur within twenty-four hours, as in monilia, while in blastomyces from seven to ten days elapse before a growth is noted; in coccidioides growth may, however, also be delayed for from two to ten days.

MOULDS

Moulds.—Of other fungi which are occasionally observed, there may be mentioned various varieties of mucor and aspergillus. Some of these organisms (*Mucor corymbifer* and *Aspergillus fumigatus*) have been found associated with cavity formation and seem to have pathogenic properties. They may at times overgrow the saprophytic bacilli (*Pneumonomycosis aspergillina* seu *mucorina*). They are best studied in the fresh specimen, not stained.

Mucor Corymbifer (Cohn): *syn.*, Lichtheimix corymbifera (Cohn). —The organism forms a snow white mycelium. The sporangiferous peduncle terminates in one or more sporangia. The sporangia are pear-shaped and measure from 15 to 70 micra in diameter; the columella is hemispherical, measuring 10 to 20 micra transversely. The spores are oval, smooth, usually measuring 2 by 3 micra, though some may be large, viz., 6.5 micra in length. The optimum temperature for growth is 36° to 37° C. It grows readily on solid media, natural or artificial (especially on Sabourand's medium), but very poorly in fluid media. It is pathogenic for laboratory animals and especially for rabbits.

Aspergillus Fumigatus (Fresenins). — The mycelium forms a greenish, bluish or grayish growth, whose component filaments measure 2 to 3 micra in diameter. The conidiophores are short

Fig. 143.—Aspergillus fumigatus. × 350. (Fränkel).

(5 micra) and terminate in a club-shaped enlargement, measuring 8 to 10 micra in diameter, bearing simple elliptical phialids (6 to 14 micras in length). These cover only half of the upper surface of the

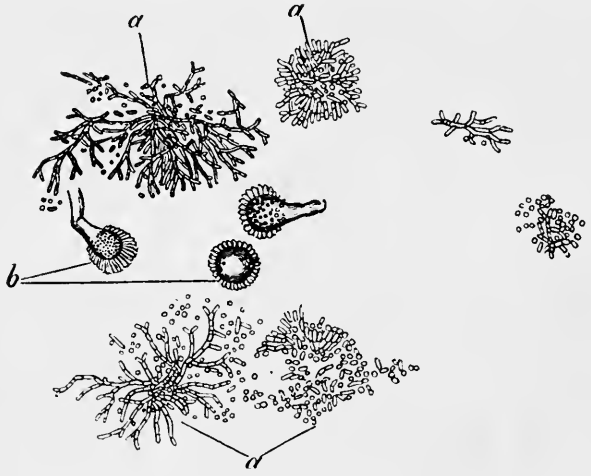


Fig. 144.—Aspergillus fumigatus of the lung, partly schematic: *a*, mycelium of aspergillus in rose-like rays; *b*, sporangium. × 285. (Weichselbaum.)

club and each carry a little chain of round smooth, rarely oval and usually colorless conidia with a diameter of 2.5 to 3 micra. As they age the reproductive structures turn yellowish, blue, green and gray.

The organism grows readily on artificial media, appearing greenish if the reaction is acid and brownish black if alkaline. Optimal temperature 37° C. It is pathogenic for the several laboratory animals. (See Figs. 143 and 144).

SARCINÆ

Sarcina Pulmonalis.—This organism has been found at times, especially in the mycotic bronchial plugs, occurring in putrid bronchitis. It is usually smaller than the Sarcina ventriculi, but larger than the variety observed in the urine; it presents the characteristic form of the latter.

Oidium Albicans.—This may be seen in children, and is usually derived from the mouth.

CRYSTALS IN SPUTUM

Of crystals which may occur in sputa, it will be necessary to consider briefly the crystals of Charcot-Leyden, hematoidin, cholesterin, margarin, tyrosin, calcium oxalate, and triple phosphates.

Charcot-Leyden Crystals.—These crystals were discovered in the sputa of patients suffering from bronchial asthma, and were supposed to stand in a causative relation to the disease. This view has been abandoned, and it is known that they may occur in other diseases as well. But while their presence is almost constant in bronchial asthma at a time when Curschmann's spirals can also be demonstrated, they are only exceptionally met with in other diseases, such as acute and chronic bronchitis, phthisis, etc. They were formerly regarded as identical with *Böttcher's sperma crystals,* but it has been shown that this is not the case. They are straight, hexagonal, double pyramids, and appear under the microscope as flattened needles of variable size (Fig. 121). Some attain a length of from 40 μ to 60 μ, while others are scarcely visible even with a comparatively high power of the microscope. They show a feeble, positive, double refraction, and have but one optical axis, while the sperma crystals are biaxial and strongly double refracting. Their behavior to solvents is essentially the same as that of the sperma crystals, but they differ from these in their insolubility in formol. They are colored yellow with Florence's reagent, while the sperma crystals are stained a bluish black. Very curiously the appearance of Charcot-Leyden crystals is closely associated with the presence of eosinophilic leukocytes, and they have hence been termed *leukocytic crystals.* They may, in fact, originate within the cells. In bronchial asthma it is not uncommon to find microscopic preparations of the sputum literally studded with eosinophilic leukocytes and free granules. Outside the sputum they are also found in the blood, in myelogenous leukemia,

and in the stools in association with animal parasites. They readily form in both normal and abnormal red bone marrow, and excellent specimens may be obtained for purposes of demonstration if a piece of a rib is allowed to remain exposed to the air for a few days. The marrow then usually contains large numbers. The crystals also form in decomposing viscera in general, and at times form a complete covering of old anatomical preparations. Their occurrence may be regarded as evidence of retrogressive changes in the cellular elements of an organ. Of the relation which they bear to the eosinophilic leukocytes, with which they are so constantly associated, nothing is known. The Charcot-Leyden crystals can be stained with the triacid stain, with thionin, with the eosinate of methylene blue, and other dyes.

Hematoidin Crystals.—These may be observed in the sputa following extravasations of blood into the lung. They frequently ocur in the form of ruby-red columns or needles; amorphous granules, however, are also seen, enclosed in the bodies of leukocytes, in which case they are probably always indicative of a previous hemorrhage, while the needles are generally observed when an abscess or empyema has perforated into the lungs. The substance is derived from blood pigment, and is now known to be identical with bilirubin.

Cholesterin Crystals.—Cholesterin crystals are at times seen in the sputa in cases of phthisis, pulmonary abscess, and, in general, whenever old accumulations of pus have entered the lung from a neighboring organ. They are readily recognized by their characteristic form and chemical properties. (See Feces.)

Fatty Acid Crystals.—These are frequently observed in cases of putrid bronchitis and gangrene of the lung, and also in cases of bronchiectasis and phthisis. They occur in the form of single needles or groups of needles, which are long and pointed. They are easily soluble in ether and hot alcohol; insoluble in water and acids. Chemically they are probably composed of the higher fatty acids, such as palmitic and stearic acids.

Tyrosin.—Crystals of this substance have been observed in cases of putrid bronchitis, perforating empyema, etc. **Leucin** is then usually also present, occurring in the form of highly refractive globules. For the recognition of these bodies, particularly of tyrosin, a chemical examination should always be made, as crystals of the soaps of fatty acids have frequently been mistaken for those of tyrosin. (See Urine.)

Calcium Oxalate Crystals.—These are rarely seen. Fürbringer observed them in large numbers in a case of diabetes, and Unger found them in a case of asthma. They are readily recognized by their envelope form and central cross, but they occur also in amorphous masses. They are soluble in mineral acids; insoluble in water, alkalies, organic acids, alcohol, and ether.

Triple Phosphate Crystals.—These are rarely seen, but may occur in cases of perforating abscesses, etc. They are recognized by their coffin-lid shape and the readiness with which they dissolve in acetic acid.

CHEMISTRY OF THE SPUTUM

In addition to the substances described, sputum may contain certain albumins, volatile fatty acids, glycogen, ferments, and various inorganic salts.

Among the albumins may be mentioned serum albumin, and especially mucin, which is often present in large amounts. While albumin is most commonly found in pneumonic and tubercular sputa, and is of a certain diagnostic value in the diagnosis of the latter condition, it should be borne in mind that any sputum containing blood will give the albumin reaction, and that the result of repeated examinations only should be considered. In pneumonic and purulent sputa albumoses also have been found.

In order to demonstrate the presence of serum albumin the sputa are treated with dilute acetic acid, when the filtrate is tested with potassium ferrocyanide, as described in the chapter on Urine. Serum albumin is, of course, found in notable quantities in cases of edema of the lungs. Especially interesting is the *albuminous expectoraion* which at times follows thoracentesis. The amount of sputum usually varies between 200 and 900 grams, but may be much larger and may reach 2000 c.c. or even more. Occasionally it begins before the tapping is completed or immediately after. More commonly, however, an interval varying from five minutes to one or two hours elapses before the expectoration begins. Its duration is variable. Sometimes it lasts only a few minutes, more often an hour or two, and in rarer cases a whole day or two. The condition is probably due to edema of the lungs.

The volatile fatty acids contained in sputa may be obtained by diluting with water, acidifying with phosphoric acid, and distilling, when the distillate is further examined as described in the chapter on Feces. Acetic, butyric, propionic, and capronic acids have been found.

Glycogen has repeatedly been demonstrated in sputa, and may be detected by Ehrlich's method. (See Blood.)

The sputa of gangrene of the lung and putrid bronchitis have been shown to contain a ferment resembling trypsin.

The myelin granules, as I have already indicated, consist largely of protagon, lecithin, and cholesterin.

CHAPTER VII

THE URINE.

GENERAL CHARACTERISTICS OF THE URINE

Appearance.—Normal urine, just voided at an ordinary temperature, is either perfectly clear or but faintly cloudy, owing to the fact that the acid and normal salts present are all soluble in water. It may be stated, as a general rule, that whenever a urine *freshly passed* presents a distinct cloudiness, some abnormality exists.

When allowed to stand for a time a light cloud develops, which gradually settles to the bottom, constituting the so-called *nubecula* of the ancients. Examined under the microscope this is found to contain a few isolated leukocytes and a few pavement-epithelial cells, derived from the bladder or genital organs. Chemically the nubecula probably consists of traces of mucus.

When kept for twenty-four hours at an ordinary temperature, crystals of uric acid are frequently observed in addition to the above elements, usually presenting the so-called whetstone form. If, however, the temperature at which the urine is kept approaches the freezing point, the entire volume becomes cloudy, owing to precipitation of acid urates, as these are much less soluble in cold than in warm water; on standing they gradually settle to the bottom of the vessel and form what is known as a *sediment*, while the supernatant fluid again becomes clear.

If kept still longer exposed to the air, at the temperature of the room, the entire volume of urine again becomes cloudy, owing to a diminution of its normal acidity, the result being a precipitation of ammonio-magnesium phosphate, calcium phosphate, and still later, when the urine has become alkaline, of ammonium urate.

Gradually a heavy sediment, containing these salts in addition to the constituents of the primitive nubecula, forms at the bottom of the vessel; the supernatant fluid, however, remains cloudy. On microscopic examination it will be seen that this cloudiness is due to the presence of enormous numbers of bacteria.

Color.—The color of normal urine may vary from a light yellow to a brownish red, the particular shade depending essentially upon the specific gravity, becoming lighter with a diminishing and darker with an increasing density. Pathologically the same rule holds good, except in diabetes, in which a very high specific gravity

is generally associated with a very light color. The reaction of the urine also exerts a marked influence upon its color, an acid urine being more highly colored than an alkaline urine, which can be readily demonstrated by allowing a specimen of acid urine to become alkaline, and by treating an alkaline urine with dilute hydrochloric or acetic acid. At the same time it may be said that every urine darkens slightly on standing, the reaction remaining acid.

A very pale urine generally indicates an excess of water, which may be normal, but may also occur in such diseases as chronic interstitial nephritis, diabetes mellitus, diabetes insipidus, hysteria, and the various anemias; it is further seen during convalescence from acute febrile diseases, while a highly colored urine, though also occurring in health, may indicate the existence of a febrile process.

Under pathological conditions the urine may be found colored by blood or biliary coloring matter. In the presence of blood the color may vary from a bright carmin to a jet black, the exact shade depending upon the quantity of blood-coloring matter present, upon changes that the blood may have undergone either before or after being passed and upon the presence of the pigment in solution or contained in red corpuscles. A reddish urine is also observed in the presence of hematoporphyrin.

Biliary pigment imparts a color which varies from a brownish yellow to a greenish brown.

Among the accidental abnormalities in color may be mentioned the smoky appearance of carbolic urine, the bright yellow due to santonin, the milky urine of chyluria, etc. In cases of hysteria or malingering, dyes may be purposely added to the urine to excite attention.

As the recognition of the causes of these various abnormalities largely depends upon a more detailed study of the individual pigments, this subject will be dealt with more fully farther on. (See Pigments and Chromogens.)

Odor.—The odor of the urine is usually of little significance. Normally it resembles that of bouillon, and in some cases that of oysters; it is probably due to the presence of several volatile acids. The odor of urines undergoing decomposition is characteristic and has been termed "the urinous odor of urine," an ill-chosen term, as this odor is always indicative of an *abnormal* condition.

The ingestion of asparagus, onions, oil of turpentine, etc., produces characteristic odors.

Consistence.—Urine, while normally fluid and but slightly viscid, may in disease acquire a marked degree of viscidity, which becomes especially apparent upon attempting its filtration; the liquid passes through the paper with more and more difficulty, and finally clogs its pores altogether. In old, neglected cases of cystitis it may be ropy and gelatinous.

Quantity.—The quantity of urine is normally subject to great variations, the amount eliminated in the twenty-four hours being influenced by that of the fluid ingested, the nature and quantity of the food, the process of digestion, the blood pressure, the surrounding temperature, sleep, exercise, body weight, sex, age, etc.

It is easy to understand, then, why figures given by different observers in different countries should vary considerably. Salkowski, in Germany, thus gives 1500 to 1700 c.c. as the normal amount; v. Jaksch, in Austria, 1500 to 2000 c.c.; Landois and Sterling, in England, 1000 to 1500 c.c.; Gautier, in France, 1250 to 1300 c.c. In the United States I have found an average secretion of from 1000 to 1200 c.c. in the adult male, and 900 to 1000 c.c. in the adult female. It is thus seen that the secretion of urine is greatest in Germany and Austria, where the body weight and ingestion of liquids are greater than in England, France, and the United States.

Children pass less, but relatively more (considering their body weight), urine than adults.

Women pass somewhat less than men.

During the summer months, when a larger proportion of water is eliminated through the skin and lungs than in cold weather, less urine is voided. The same occurs during repose, more urine being passed during active exercise, and hence less during the night than during the day.

The amount of urine secreted in the different hours of the day varies greatly, reaching its maximum a few hours after meals. It decreases toward night, and reaches its lowest point in the first hours of the night, after which it begins to rise rapidly until 2 or 3 o'clock in the morning.

The ingestion of large amounts of liquid, of course, increases the daily amount considerably, and 3000 c.c. may be passed under such conditions by an individual in good health, while it may decrease to 800 or 900 c.c. when but little liquid is taken.

After the ingestion of much solid food the secretion of urine is temporarily diminished.

Pathologically the amount of urine varies within wide limits. On the one hand there may be diminution (oliguria), which may go to the point of complete anuria, or there may be an increased flow (polyuria), amounting to many liters.

Polyuria.—This is seen especially in diabetes mellitus and insipidus, in connection with the resorption of large effusions, during convalescence from acute febrile diseases (epicritic polyuria), in chronic interstitial nephritis, early in the course of renal tuberculosis, in myelomatosis, in various diseases of the nervous system (tabes, paresis, brain tumors), hysteria, etc. *Oliguria* is, on the whole, more frequent than polyuria; it is common in connection with all febrile conditions, in acute and chronic parenchymatous nephritis,

in cases of cardiac insufficiency from whatever cause, when there is loss of fluid from the body as the result of diarrhœa or vomiting, in connection with obstruction to the flow of blood in the vena cava or liver (atrophic hepatic cirrhosis, acute yellow atrophy, thrombosis of the vena cava and the renal vein), in eclampsia, lead colic, hysteria, etc.

Specific Gravity.—The specific gravity of normal urine varies between 1.015 and 1.025, corresponding to 1200 to 1500 c.c., viz., the normal amount of urine voided in twenty-four hours. Pathologically, a specific gravity of 1.002 on the one hand and 1.060 on the other may occur, depending upon the amount of solids and fluids present, increasing as the solids increase, the amount of urine remaining the same and decreasing as the amount of fluid increases, the solids remaining the same. The specific gravity is thus an index in a general way of the metabolic processes taking place in the body.

The necessity of determining the specific gravity of the total amount of urine voided in a given case, and not that of an individual specimen passed during the twenty-four hours, becomes apparent upon considering the variations which may occur in the quantity of solids and liquids ingested during the day. The ingestion of large amounts of fluid would, of course, result in the passage of a correspondingly large quantity of urine within the next few hours, containing but a small amount of solids, and hence presenting a low specific gravity. From such an observation it would be erroneous to infer a diminished excretion of solids for the day, as succeeding specimens would in all probability be passed presenting a higher specific gravity. *An observation made upon a specimen taken from the collected urine of the twenty-four hours, moreover, can only then convey a correct idea if the total quantity is known.*

From the specific gravity the amount of solids can be calculated with sufficient accuracy for clinical purposes by multiplying the last two decimal points by 2, the number obtained indicating the amount of solids in 1000 c.c. of urine.

From the rule that the specific gravity of a urine is inversely proportionate to the amount of fluid eliminated, it must follow that whatever causes produce oliguria will also produce a high specific gravity, while all those causes which produce polyuria will similarly produce a low specific gravity, with the following exceptions:

1. A diminished amount of urine with a lowered specific gravity occurs in many chronic diseases and toward the fatal termination of acute diseases.
2. The same may be observed in certain cases of edema,
3. Following copious diarrhea, vomiting, and sweating.
4. A high specific gravity is associated with polyuria in diabetes mellitus.

Unfortunately the determination of the specific gravity and the solids contained in urine does not furnish as valuable information

in many cases as would be expected *a priori*. This is largely owing to the fact that the organic constituents of the urine have a lower specific gravity than the inorganic salts, and especially the chlorides, which are usually present in considerable amount. It thus not infrequently happens that the nitrogenous constituents are considerably increased, while the specific gravity is relatively low, owing to the absence or a diminution in the amount of chlorides. In other words, while the specific gravity may be regarded as a fair index of the total amount of solids excreted, its increase or decrease furnishes no information as to the nature of the constituents causing such a change.

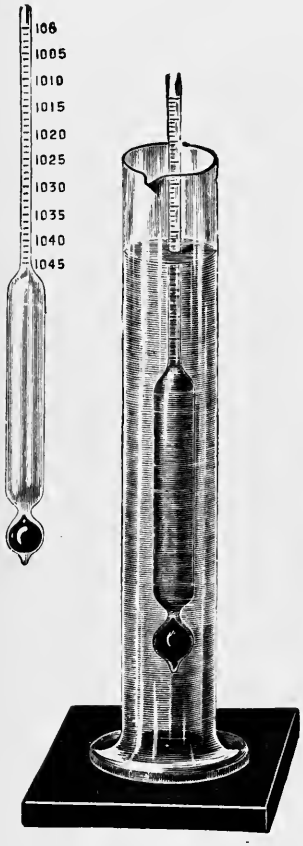


Fig. 145.—Urinometer.

Determination of Specific Gravity.—The specific gravity of the urine is most conveniently determined by means of a hydrometer indicating degrees varying from 1.002 to 1.040. Such instruments, constructed especially for the examination of urine, are termed *urinometers* (Fig. 145). A good instrument should have a stem upon which the individual divisions are at least 1.5 mm. apart, and each division should correspond to 0.5 degree.

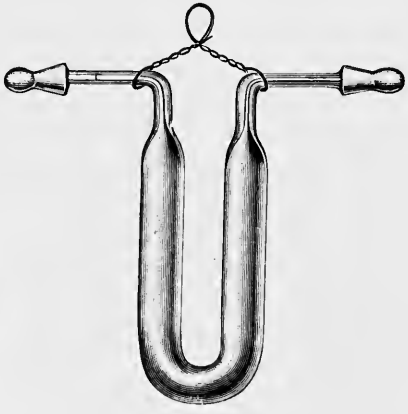


Fig. 146.—The pyknometer.

Urinometers may also be purchased which are provided with a thermometer. Every instrument should be carefully tested by comparison with a *standard* hydrometer.

In order to determine the specific gravity in a given case a cylindrical vessel is nearly filled with urine and the urinometer *slowly*

introduced, the reading being taken at the lower meniscus as soon as the instrument has come to rest.

Precautions: 1. The urinometer must be given ample room, and the reading should never be taken when the instrument touches the sides of the vessel, as owing to capillary attraction it is otherwise raised, causing the reading to be too high.

2. The instrument must be perfectly dry and clean before being used, and should never be allowed "to drop" into the urine, as otherwise the weight of the instrument is increased by adhering drops of fluid and the reading is too low.

3. Any foam upon the surface of the urine should first be removed by means of a piece of filter paper, as it interferes with the accuracy of the reading; bubbles of air adhering to the instrument, and thereby elevating it, should be removed with a feather.

4. The specific gravity should always be determined in specimens taken from the twenty-four-hour urine.

Determination of the Solid Constituents.—As indicated above, the amount of solids can be calculated with a degree of accuracy sufficient for clinical purposes by multiplying the last two figures of the specific gravity by 2; the number obtained indicates the amount of solids in every 1000 c.c. of urine. If greater accuracy is required, the following method may be employed: 5 c.c. of urine, accurately measured, are placed in a watch crystal containing a little dry sand (sand and crystal having been previously weighed); this is placed over a dish containing concentrated sulphuric acid, and under the receiver of an air pump which has been made perfectly air-tight by thoroughly lubricating the ground-glass edge of the bell with mutton tallow and applying the bell with a slightly grinding movement to the ground-glass plate. The receiver is now exhausted and the urine allowed to remain in the vacuum for twenty-four hours, when the bell is again exhausted and left for twenty-four hours longer; at the end of this time the crystal is weighed, the difference between the two weights obtained indicating the amount of solids in 5 c.c. of urine, from which the percentage and total amount are readily calculated.

The slight loss of ammonia which results when this method is employed scarcely affects the accuracy of the result.

Reaction.—The reaction of the twenty-four-hour urine is, as a rule, acid; individual specimens, passed in the course of the same twenty-four hours, may be either alkaline, acid, or amphoteric.

It has been generally held in the past that the acid reaction of normal urine is due to the presence of diacid phosphates. But it was assumed also that monacid phosphate was present at the same time. Folin has shown that this assumption is not correct, that the phosphates in clear urine are all of the diacid kind, and

that the acidity of such urines is ordinarily greater than the acidity of all the phosphates, the excess being due to free organic acids.

An alkaline urine results when the alkalies exceed the acid equivalents in amount. An amphoteric urine (red litmus turned blue and blue litmus red) is the outcome, when the acid equivalents of diacid phosphates equal the basic equivalents of the monacid phosphates; this is essentially an accidental occurrence.

As the alkalinity of the blood increases the acidity of the urine decreases, until an alkaline urine results. The degree of the alkalinity of the blood, however, depends essentially upon the nature of the food and the secretion of the gastric juice, viz., the hydrochloric acid. The ingestion of vegetable food, rich in salts of organic acids, which become oxidized in the body to the carbonates of the alkalies, will result in the passage of an alkaline urine. In the case of animal food the reverse holds good. The alkaline carbonates here formed are not sufficient to neutralize the excess of acids, and diacid phosphate of sodium is hence eliminated in large quantity.

As the alkalinity of the blood is increased during the secretion of the acid gastric juice, it may happen, especially following the ingestion of a large amount of food, that an alkaline urine is voided. If this does not take place, the acidity of the urine is at least diminished, but increases again during the process of resorption.

If an acid urine is allowed to stand exposed to the air for a certain length of time, its degree of acidity gradually diminishes and the reaction finally becomes alkaline. At the same time the urine becomes cloudy and deposits a sediment which consists of ammoniomagnesium phosphate, $MgNH_4PO_4 + 6H_2O$, neutral calcium phosphate, $Ca_3(PO_4)_2$, and still later contains ammonium urate, $C_5H_2NH_4)_2N_4O_3$, in addition to the constituents of the primitive nubecula—*i. e.*, a few leukocytes and pavement-epithelial cells. The entire volume of urine, moreover, remains cloudy, owing to the presence of innumerable bacteria. The odor becomes extremely disagreeable and distinctly "urinous." In short, "ammoniacal decomposition" has occurred. This has been shown to depend upon the action of certain bacteria, notably the Micrococcus ureæ and the Bacterium ureæ.

An alkaline urine, the alkalinity of which is not due to ammoniacal fermentation, however, but to other causes, as indicated above, may, of course, undergo the same change as an acid urine; but it is necessary to distinguish sharply between these two varieties of alkaline urines, as the recognition of the cause of the alkalinity is very often most important in diagnosis. The distinction is readily made by fastening a piece of sensitive red litmus paper in the cork of the bottle containing the urine. If the alkalinity of the urine is due to the presence of ammonia, the litmus paper will turn blue, but soon changes to red when exposed to the air; while a urine the

alkalinity of which is due to the presence of fixed alkalies will turn red litmus paper blue *only when immersed in the urine*, the change in color at the same time persisting.

As ammoniacal decomposition can also occur within the urinary passages, it is important, whenever an alkaline reaction due to the presence of ammonia is observed, to test the urine at once upon being voided, or, still better, to procure a portion with a catheter. Such urines are frequently seen in neglected cases of cystitis the result of paralysis, prostatic disease, etc.

An intensely acid reaction is observed in almost all concentrated urines, especially in fevers, in certain diseases of the stomach associated with a diminished or suspended secretion of hydrochloric acid, in gout, lithiasis, acute articular rheumatism, chronic Bright's disease, diabetes, leukemia, scurvy, etc. Whenever a very acid urine is secreted for a considerable length of time the possibility of renal irritation and the formation of concretions should be borne in mind.

An alkaline urine the alkalinity of which is not owing to the presence of ammonia, but to fixed alkali, is observed in certain cases of debility, especially in the various forms of anemia, following the resorption of alkaline transudates, the transfusion of blood, frequent vomiting, a prolonged cold bath, etc. It may also be due to the ingestion of certain drugs, viz., salts of the organic acids and alkaline carbonates, the former being transformed into the latter, as has been mentioned. An increase in the degree of acidity may similarly take place after the ingestion of mineral acids.

Of interest is the observation of Pick that in twenty-four to forty-eight hours after the crisis in pneumonia the urine shows a marked decrease in its acidity, becoming neutral or even alkaline. This phenomenon, which was observed in 31 out of 38 cases, persists for a day or a day and a half, and then the acidity returns. In all likelihood the change is due to absorption of the large amounts of sodium which are present in the exudate.

An increase in the acidity of the urine upon standing has repeatedly been observed, and is probably due to the formation of new acids from preëxisting acid-yielding substances, such as certain carbohydrates, alcohol, etc., which have undergone fermentation. This phenomenon is frequently observed in diabetic patients.

A decrease in the acidity of normal urine upon standing, however, is the rule, owing to a gradual decomposition of sodium urate by the acid sodium phosphate, acid sodium urate, and, later on, uric acid resulting, which are thrown down as a sediment in consequence of the diminished acidity of the urine, and which, hence, no longer influence its reaction.

Determination of the Acidity of the Urine.—Folin's Method.—The total acidity which indicates the acidity due to diacid phosphates and free organic acids is first determined as follows: 25 c.c. of urine

are treated with 1 or at most 2 drops of 0.5 per cent. alcoholic solution of phenolphthalein and 15 to 20 grams of powdered potassium oxalate. The solution is shaken for about a minute and titrated *at once* with decinormal sodium hydrate solution until a faint yet distinct pink color is obtained. The flask should be shaken during the titration, so as to keep the solution as strong as possible in oxalate. The acidity is expressed in terms of decinormal sodium hydrate solution for the total amount of urine of twenty-four hours. The total acidity is termed T.

In a second specimen the total phosphates are then determined, the value being termed P. (See Phosphates.) The result is expressed in terms of decinormal acid, viz., alkali as above (1 c.c. $\frac{n}{10}$ = 7.1 mg. of P_2O_5). T minus P then indicates the acidity due to uncombined organic acids (O. A.).

It may happen that the acidity calculated from the total phosphates is greater than the titrated acidity; in that case practically no free organic acids are present and the titrated acidity represents the amount of phosphates present in the diacid form. Urines of this kind are turbid, unless they are also free from calcium (Folin).

As average normal value for the acidities of the total bulk of twenty-four hours' urine Folin obtained 617 (c.c. $\frac{1}{10}$ n. acid), of which 304 was referable to mineral and 313 to organic acidity. The corresponding minimal and maximal values were T. 554 and 669; M. A. 204 and 417; O. A. 252 and 378.

With this method a complete revision of all the work previously done will be necessary. The older results have reference only to the old method of titration with one-tenth normal solution of sodium hydrate.

Determination of the Mineral Acidity or the Excess of Mineral Acids or Bases.—Folin's method may be employed instead of determining all the different metals and acids separately, as Bunge, Magnus-Levy, and others have done.

To 25 c.c. of urine in a platinum dish is added from 0.3 to 0.5 gram of potassium carbonate, weighed within an accuracy of two-tenths of a mg. The solution is evaporated to dryness, and the residue ignited, when perfectly dry, over a radial burner, using at first a very low heat, and at no time allowing the dish to become more than faintly red hot. The dish is heated at this temperature for one hour, then cooled, when 10 c.c. of hydrogen peroxide are added and evaporated. The dried residue is ignited as before for one hour. It is dissolved in an excess of tenth normal hydrochloric acid and water (50 to 75 c.c. $\frac{n}{10}$ HCl), transferred to an Erlenmeyer flask, boiled to remove carbonic acid, and cooled. One or two drops of phenolphthalein solution and a few crystals of neutral potassium oxalate (to precipitate the calcium) are added, and the solution titrated as usual. The ammonia, the acidity of the hydrogen per-

oxide, and the acidity of the organic sulphur (neutral and ethereal, 8 grams of which are taken to represent 1 c.c. tenth normal acid) must be subtracted from the result given by the direct titration. These values, as well as the acidimetric value of the potassium carbonate, must be separately determined.

This procedure gives very reliable results, if proper care is used in the evaporation and the burning of the urine. It is to be used only when the actual excess of mineral acids above that necessary for the neutralization of the mineral bases is to be estimated, or when the total amount of organic acids in urine (whether free or combined with bases) is to be determined (Folin).

CHEMISTRY OF THE URINE

General Chemical Composition of the Urine.—A general idea of the chemical composition of the urine and the quantitative variations of the individual components may be formed from the following table, which I have constructed from analyses made in my laboratory. The individuals from whom the urines were obtained were adults, and their general mode of life, as regards diet, exercise, etc., was that of the average American city dweller. In addition, the following substances may be encountered under pathological conditions: serum albumin, serum globulin, albumoses, mucin (nucleoalbumin), glucose, lactose, inosit, dextrin, biliary constituents, viz., bile acids and bile pigments, blood pigments, melanin, leucin tyrosin, oxybutyric acid, allantoin, fat, lecithin, cholesterin, acetone, alcohol, urocaninic acid, cystin, hydrogen sulphide, and still others.

Analysis of Urine

Water	1200 to 1700 grams.
Solids	60.0 "
Inorganic solids	25.0 to 26.0 "
Sulphuric acid (H_2SO_4)	2.0 to 2.5 "
Phosphoric acid (P_2O_5)	2.5 to 3.5 "
Chlorine (NaCl)	10.0 to 15.0 "
Potassium (K_2O)	3.3 "
Calcium (CaO)	0.2 to 0.4 "
Magnesium (MgO)	0.5 "
Ammonia (NH_3)	0.7 "
Fluorides, nitrates, etc.	0.2 "
Organic solids	20.0 to 35.0 "
Urea	10.0 to 30.0 "
Uric acid	0.2 to 1.0 "
Xanthin bases	1.0 "
Kreatinin	0.05 to 0.08 "
Oxalic acid	0.05 "
Conjugate sulphates	0.12 to 0.25 "
Hippuric acid	0.65 to 0.7 "
Volatile fatty acid	0.05 "
Other organic solids	2.5 "

Quantitative Estimation of the Mineral Ash of the Urine.—In order to estimate the amount of mineral ash in the urine the following method may be employed: 50 c.c. of urine are evaporated to dryness in a weighed porcelain dish, at a temperature of 100° C., and then heated, while covered, over the free flame until gases cease to be evolved, care being taken not to heat too strongly, in order to avoid sputtering. The residue is taken up with distilled boiling water, and, after standing, filtered through a Schleicher and Schüll filter, the weight of the ash of which is known. The dish and the contents of the filter are well washed with hot water. Filtrate and washings are set aside and the dish and filter dried in the oven at 115° C. The filter is now placed in the dish and slowly incinerated. As soon as the ash has turned white the filtrate and washings are placed in the same dish, evaporated at 100° C., and then carefully heated over the free flame. Upon cooling in the desiccator (Fig. 147) the dish with its contents is weighed, the difference between its present and previous weight indicating the quantity of ash contained in 50 c.c. of urine.

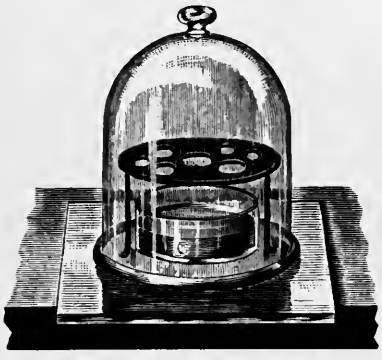


Fig. 147.—Desiccator.

Precautions: 1. Care should be taken to allow the dish to become faintly red only for a moment, as some of the chlorine is otherwise volatilized. Some phosphoric acid may also escape, and too strong a heat, moreover, may cause the transformation of sulphates into sulphides, the organic material present acting as a reducing agent.

2. If the organic ash is not completely incinerated, it is best to allow the dish to cool and then to moisten the ash with a few drops of dilute sulphuric acid, when the heating is continued.

THE CHLORIDES

The chlorides which are excreted in the urine are derived from the food. As they are thus present in a much larger amount than all other inorganic salts combined, and in quantity more than sufficient to supply the needs of the body economy, the relatively large amount of chlorides found in the urine under physiological conditions, as compared with the other inorganic constituents, is readily explained.

Of the alkalies in the urine, sodium in combination with chlorine exists in greatest amount, and for clinical purposes it is most convenient to calculate the total quantity of chlorides in terms of sodium

chloride; a small proportion also occurs combined with potassium, ammonium, calcium, and magnesium.

From 11 to 15 grams of sodium chloride, representing the total quantity of chlorine, are normally eliminated in the twenty-four hours, the amount depending, of course, directly upon that contained in the food ingested.

Pathologically the excretion of the chlorides may vary within wide limits, diminishing on the one hand to zero and increasing on the other to 50 grams or more in the twenty-four hours. The lowest values are met with in pneumonia, where the chloride reaction may disappear entirely. The condition is not pathognomonic of the disease in question, however, but may be observed, even though to a less marked extent, in many of the acute febrile diseases, such as scarlatina, measles, smallpox, typhus and typhoid fever, recurrens, and acute yellow atrophy. In malarial fever the diminution is less marked. Low values are further noted in all acute and chronic renal diseases, in cancer of the stomach, in chronic hypersecretion associated with dilatation, in anemic conditions, rickets, melancholia, and idiocy, in pemphigus foliaceus, in the beginning of impetigo, and in chronic lead poisoning.

The chlorides are found in *increased* amount in all conditions in which retention has previously occurred, chief among these being the acute febrile diseases and cases in which a resorption of exudates and transudates, associated with an increased diuresis, is taking place. A marked increase has been noted in some cases of diabetes insipidus, in which 29 grams have been eliminated in the twenty-four hours. A similar increase may occur in prurigo, in which, in one instance, 29.6 grams were passed in twenty-four hours. In cases of general paresis, during the first stage, an increased elimination goes hand in hand with an increased ingestion of food. In epilepsy the polyuria following the attacks is associated with an increase in the chlorides.

Of drugs, certain diuretics, and some of the potassium salts produce an increase: the chlorine contained in chloroform, whether administered internally or as an anesthetic, is in part excreted in the form of a chloride. Salicylic acid, on the other hand, is said to cause a temporary diminution.

It is of practical importance to note that in acute febrile diseases the diminution in the chlorides appears to vary with the intensity of the disease, a decrease to 0.05 gram pro die justifying the conclusion that the case under observation is of extreme gravity. It may at times also indicate a preceding attack of severe diarrhea or the formation of exudates of considerable extent. A continued increase, on the other hand, should lead to the conclusion that the patient's condition is improving.

The elimination of the chlorides also furnishes a fair index to the digestive powers of the patient. All other causes which might lead

to an increase or decrease being eliminated, an excretion of from 10 to 15 grams indicates a fair condition of the appetite and a normal digestive power, a decrease being associated with the reverse.

An increased elimination of chlorides occurring in cases of edema, and associated with the existence of serous exudates, is always of good prognostic omen, pointing to a resorption of the fluid.

A continued elimination of more than 15 to 20 grams, all other causes being excluded, may be considered as pathognomonic of diabetes insipidus.

Of late, attention has been directed to the ratio between the elimination of the chlorides and the total nitrogen. With an ordinary diet this is as 1 to 1 (Salkowski), even though the total amount of chlorides may not amount to 10 to 15 grams, but may be as low as 7 to 10 grams. In disease this ratio may be much disturbed owing to chloride retention (1 Cl to 15 N); a change toward the normal is *cæteris paribus* a favorable sign.

Test for Chlorides in the Urine.—The recognition of the chlorides in the urine is based upon the fact that silver nitrate causes their precipitation. The silver chloride thus formed is insoluble in nitric acid.

The test is made in the following manner: A few cubic centimeters of the urine are acidified in a test-tube with about 10 drops of pure nitric acid, and treated with a few cubic centimeters of silver nitrate solution (1 to 20). The occurrence of a white precipitate indicates the presence of chlorides. An idea may be formed at the same time of the quantity present; the occurrence of a heavy, caseous precipitate points to a large amount. Albumin, if present, must first be removed by boiling, after acidifying the urine with a few drops of dilute acetic acid.

Quantitative Estimation of the Chlorides by the Method of Salkowski-Volhard.—When a solution of silver nitrate acidified with nitric acid is treated with a solution of potassium sulphocyanide or ammonium sulphocyanide, in the presence of a ferric salt, the potassium sulphocyanide first causes the precipitation of white silver sulphocyanide, which, like silver chloride, is insoluble in nitric acid. As soon as every trace of silver is precipitated, it combines with the ferric salt to form ferric sulphocyanide, which is of a blood-red color. If the potassium sulphocyanide solution is of known strength, it is possible to estimate accurately the amount of silver present in the solution, the ferric salt serving as an indicator of the end of the reaction between the silver and the potassium sulphocyanide.

Application to the urine: to urine which has been acidified with nitric acid an excess of a silver solution of known strength is added, and the silver not used in the precipitation of the chlorides then estimated as indicated above. The difference between the quantity thus found and the total amount used will be that consumed in the pre-

cipitation of the chlorides, from which, knowing the strength of the silver solution, its equivalent in terms of sodium chloride is readily determined.

Reagents Required.—1. A solution of silver nitrate of such strength that each cubic centimeter shall correspond to 0.01 gram of sodium chloride.

2. A solution of potassium sulphocyanide of such strength that 25 c.c. shall correspond to 10 c.c. of the silver nitrate solution.

3. A solution of a ferric salt, such as ammonioferric alum, saturated at ordinary temperature.

4. Nitric acid (specific gravity, 1.2).

Preparation of these solutions:

1. As pointed out, the silver nitrate solution is made of such strength that each cubic centimeter shall correspond to 0.01 gram of sodium chloride.

The silver nitrate must be pure, and it is best to use the crystallized salt, and not sticks wrapped in paper, which always contain reduced silver. In order to test the purity of the salt, about 1 gram is dissolved in distilled water, heated to the boiling point, the silver precipitated by dilute hydrochloric acid and filtered off. When evaporated in a platinum crucible the filtrate should leave either no residue at all or only a very faint one; otherwise it is necessary to recrystallize the salt until the desired degree of purity is reached.

The determination of the quantity to be dissolved in 1000 c.c. of water is based upon the fact that 1 molecule of silver nitrate (molecular weight 170) combines with 1 molecule of sodium chloride (molecular weight 58.5) to form silver chloride and sodium nitrate. As the solution of silver nitrate shall be of such strength that 1 c.c. corresponds to 0.01 gram of sodium chloride, or 1000 c.c. to 10 grams, the quantity to be dissolved in 1000 c.c. is found according to the following equation:

$$58.5 : 170 :: 10\ x,\ 58.5\ x = 1700,\ x = 29.059.$$

Theoretically, then, this quantity should be dissolved in 1000 c.c. of water. It is better, however, to dissolve it in a quantity somewhat less than 1000 c.c. such as 900 or 950 c.c., as the silver salt contains water of crystallization and the weighed-off quantity would not represent the exact amount required, but less, the correcting of a solution which is too strong being a much simpler matter than that of a solution which is too weak.

To make this correction, or, in other words, to bring the solution to its proper strength, 0.15 gram of sodium chloride, which has previously been dried carefully by heating in a platinum crucible, is accurately weighed off, dissolved in a little distilled water, and further diluted to about 100 c.c. To this solution a few drops of a solution

of potassium chromate are added, when the mixture is titrated with the silver solution. The silver nitrate will first precipitate the sodium chloride, and then combine with the potassium chromate, forming red silver chromate. The slightest orange tint remaining after stirring indicates the end of the reaction. Were the solution of the silver nitrate of the proper strength, exactly 15 c.c. should have been used, as each cubic centimeter shall represent 0.01 gram of sodium chloride. As a matter of fact, less will in all probability be needed, the solution having been purposely made too strong. Its correction then becomes a simple matter, as it is merely necessary to determine the degree of dilution required.

Supposing that 29.059 grams of silver nitrate were dissolved in 950 c.c. of water, and that 14.5 c.c. instead of 15 c.c. had been required to precipitate the 0.15 gram of sodium chloride, it is evident that each 14.5 c.c. of the remaining solution must be diluted with 0.5 c.c. of water. It is, hence, only necessary to divide the number of cubic centimeters of the silver nitrate solution remaining by 14.5; the result multiplied by 0.5 represents the amount of water which must be added in order to bring the solution to the required strength.

In the example given the necessary correction would be:

$$C = \frac{935.5 \times 0.5}{14.5} = 32.25$$

32.25 c.c. of distilled water would have to be added to the remaining 935.5 c.c. If the solution is found too weak, it is best to make it too strong, and then to correct as described.

2. Preparation of the potassium sulphocyanide solution: As 1 molecule of silver nitrate (molecular weight 170) combines with 1 molecule of potassium sulphocyanide (molecular weight 97), the quantity of the latter to be dissolved in 1000 c.c. of water is found from the following equation:

$$170 : 97 :: 11.6236 : x;\ 170\,x = 11.6236 \times 97;\ x = 6.6.$$

As potassium sulphocyanide is extremely hydroscopic, a solution is made which is too strong, by dissolving about 10 grams of the salt in 900 c.c. of distilled water. In order to bring this solution to its proper strength, 10 c.c. of the silver solution are diluted to 100 c.c.; 4 c.c. of nitric acid (specific gravity 1.2) and 5 c.c. of the ammonio-ferric alum solution are added, when the mixture is titrated with the potassium sulphocyanide solution; the end reaction is recognized by the production of a slightly reddish color, which persists on stirring. The sulphocyanide solution having been purposely made too strong, it will be found that less than 25 c.c. are needed to precipitate all the silver present. The quantity of water necessary for dilution is then ascertained by a simple calculation (see above).

3. The solution of ammonioferric alum is a solution saturated at ordinary temperatures, care being taken to insure the absence of chlorides in the salt, which may be effected, if necessary, by recrystallization.

Method as Applied to the Urine.—Ten c.c. of urine are placed in a small stoppered flask bearing a 100 c.c. mark, diluted with 50 c.c. of distilled water, and acidified with 4 c.c. of nitric acid. From a burette, 15 c.c. of the standard solution of silver nitrate are added. The mixture is thoroughly agitated and diluted with distilled water to the 100 c.c. mark. The silver chloride formed is filtered off through a *dry*, folded filter into a *dry* graduate; 80 c.c. of the filtrate are placed in a beaker, and, after the addition of 5 c.c. of the ammonioferric alum solution, titrated with the sulphocyanide solution until the end reaction—*i. e.*, a slightly reddish tinge—is seen. If necessary, two such titrations should be made, the sulphocyanide solution being added 1 c.c. at a time in the first, while in the second the total number of cubic centimeters needed to bring about the end reaction, less 1 c.c., are added at once, and then 0.1 c.c. at a time.

The amount of chlorides present in the urine is calculated as follows:

Example.—Total quantity of urine 600 c.c.; 6.5 c.c. of the sulphocyanide solution were required to bring about the end reaction in 80 c.c. of the filtrate; this would correspond to 8.125 c.c. for the total 100 c.c. of filtrate, representing 10 c.c. of urine, as is seen from the equation

$$n : 80 : : x : 100;\ 80\,x = 100\,n;\ x = \frac{100\,n}{80} = \frac{5\,n}{4},$$

in which x represents the number of cubic centimeters corresponding to 100 c.c. of the filtrate, and n the number of cubic centimeters actually used.

These 8.125 c.c. were used in precipitating the silver nitrate not decomposed by the chlorides. As 25 c.c. of the sulphocyanide solution correspond to 10 c.c. of the silver solution, the excess of silver solution in cubic centimeters is found from the equation

$$25 : 10 : : N : x;\ 25\,x = 10\,N;\ x = \frac{10\,N}{25} = \frac{2\,N}{5}$$

in which x represents the excess of the silver solution in cubic centimeters, and N that of the sulphocyanide solution as found according to the equation above, x in this case being 3.25 c.c.

The difference between the total amount of silver solution employed (*i. e.*, 15 c.c.) and the excess (*i. e.*, 3.25 c.c.) indicates the number of cubic centimeters necessary for the precipitation of the chlorides in 10 c.c. of urine. In the case under consideration

11.75 c.c. were employed. As 1 c.c. of the silver solution represents 0.01 gram of sodium chloride, there must have been present in the 10 c.c. of urine 0.1175 gram; in 100 c.c., hence, 1.175 grams, and in the total amount—*i. e.*, 600 c.c. of urine—7.05 grams.

The method described may be employed in the presence of albumins, albumoses, and sugar; the urine, however, must be fresh, so as to insure the absence of nitrous acid.

THE PHOSPHATES

The phosphates occurring in the urine are sodium, potassium, calcium, and magnesium salts of the tribasic acid H_3PO_4. The most important of these, as was pointed out in the chapter on Reaction, is the diacid sodium phosphate NaH_2PO_4, to which the acidity of the urine is in part due. It is owing to the presence of this salt in the urine that the calcium phosphate is held in solution; the fact, at least, that calcium and magnesium phosphate are thrown down when the urine is neutralized would point to this conclusion.

The character of the phosphates is liable to considerable variation, depending upon the degree of acidity of the urine. As would be expected, diacid sodium phosphate and diacid calcium phosphate are present in an acid urine; in an amphoteric urine, in addition to these there are found disodium phosphate, monocalcium phosphate, and monomagnesium phosphate, while in an alkaline urine, disodic phosphate, trisodic phosphate, neutral calcium phosphate, and neutral magnesium phosphate may be present.

The alkaline phosphates normally exceed the earthy phosphates by one-third, and sodium is combined with by far the greater amount of phosphoric acid, the potassium salt normally occurring in only very small amounts.

In addition to the mineral phosphates, phosphoric acid is excreted also in combination with glycerin as glycerin-phosphoric acid, which need not, however, be considered in a quantitative estimation, as it is present only in traces.

As in the case of the chlorides, the greater portion of the phosphates is derived from the food, while only a small portion is referable to the tissue proteins. Not all the phosphoric acid ingested, however, is excreted in the urine, as one-third to one-fourth of the total quantity is eliminated in the feces.

The quantity of phosphoric acid, which normally varies between 2.5 and 3 grams, is thus largely dependent upon the amount ingested, increasing with an animal and decreasing with a vegetable diet.

In disease the total amount of phosphates may either be increased or diminished.

A *diminished* elimination is observed in most acute febrile maladies, the degree of diminution being usually proportionate to the severity

of the disease, reaching its lowest figure as death approaches; further, in acute and to some extent also in chronic nephritis, amyloid degeneration, in the various anemias, in osteomalacia during attacks of major hysteria, in chronic lead poisoning, in Addison's disease, acute yellow atrophy, in certain cases of hepatic cirrhosis, in gout (before the onset of acute symptoms), etc. An *increased* elimination, on the other hand, is less common. Teissier speaks of a phosphatic diabetes with values up to 9 grams, figures which are approached only in pseudoleukemia, leukemia, and hemorrhagic purpura (5 to 15 grams). In true diabetes high values of P_2O_5 may occur at times when the sugar values are low, and *vice versa*.

While important conclusions cannot be drawn from a knowledge of the absolute phosphatic elimination, a study of the *relative phosphatic excretion* seems to promise more valuable results. According to Zülzer, a definite quantity of phosphates and of the urinary nitrogen is referable to the destruction of albuminous material, so that the relation between the phosphoric acid and the nitrogen must be constant. Another portion is derived from lecithin, one of the most important constituents of nerve tissue, which contains more phosphorus than the albuminous molecule. Whenever, then, the lecithin-containing tissues are more involved in the general metabolism than under normal conditions the relation will no longer be a stable one. This relation which exists between the elimination of nitrogen and phosphoric acid has been termed the *relative value* of phosphoric acid.

The relative value of phosphoric acid in the urine has been found to vary from 17 to 20, that of the blood being 3, of muscle tissue 12.1, of brain 44, of bone 426 to 430. This value supposes the absolute value to vary between 2 and 3 grams pro die. It is found according to the following equation:

$$N : P_2O_5 : : 100 : x; \text{ and } x = \frac{100 \times P_2O_5}{N},$$

in which N indicates the amount of nitrogen actually observed, P_2O_5 the amount of phosphoric acid in the same specimen of urine, and x the amount of P_2O_5 coresponding to 100 grams of N. By observing this relative value a much better idea may be formed of the metabolic processes taking place in the body in disease than from a mere expression of the absolute phosphatic value.

In acute febrile diseases the relative as well as the absolute diminution of the phosphates has been ascribed to a retention, they being possibly utilized in the building up of white blood corpuscles. In the course of these diseases oscillations in the relative value are frequently observed; during convalescence the relative as well as the absolute value again rises.

In accordance with these considerations a diminished relative excretion of phosphoric acid should be expected in all cases associated with a notable elimination of leukocytes through other channels, as in pneumonia, for example, or a storing away of the same, as in cases of empyema. The facts observed are in accord with this view.

A relative decrease has further been noted in the various forms of anemia, conditions of cerebral excitation, and especially preceding an attack of epilepsy. In progressive paralysis following syphilis the relative value, at first low, rises greatly after the administration of potassium iodide, while the excretion of the earthy phosphates is lessened. In chronic cerebral affections, delirium tremens, and acute hydrocephalus a relative decrease has been noted. In mania, during the period of excitement, both the alkaline and the earthy phosphates are found increased, while during the stage of depression, as also in melancholia, the alkaline phosphates are diminished and the earthy phosphates increased. On the other hand, an increase in the relative value has been noted in apoplexy (amounting to 34.3 in one case, two days after an attack), brain tumors, tabes, arthritis deformans (30), pernicious anemia (23.8 to 58), etc.

Quantitative Estimation of the Total Amount of Phosphates.—Principle.—When a solution of disodium phosphate acidified with acetic acid is treated with a solution of uranyl nitrate or uranyl acetate, a dirty-looking precipitate of uranyl phosphate is thrown down. It is apparent that the quantity of phosphoric acid can be estimated accurately if the solution of uranyl nitrate or acetate is of known strength.

Solutions Required.—1. A solution of uranium nitrate of such strength that 20 c.c. shall correspond to 0.1 gram of P_2O_5.

2. A solution containing sodium acetate and acetic acid.

3. Tincture of cochineal.

Preparation of these solutions:

1. From the equation

$$2UO.NO_3 + Na_2HPO_4 = (UO)_2HPO_4 + 2NaNO_2$$

it is apparent that 2 molecules of uranium nitrate combine with 1 molecule of disodium phosphate to form uranium phosphate and sodium nitrate. The molecular weight of uranium nitrate being 318 and that of disodium phosphate 142, it is seen that 636 parts by weight of the former combine with 142 parts by weight of the latter.

As 20 c.c. of the solution of uranium nitrate shall correspond to 0.1 gram of P_2O_5, 1000 c.c. must be equivalent to 5 grams of P_2O_5. In 142 parts by weight of disodium phosphate there would be present 71 grams of P_2O_5, equivalent to 636 parts by weight of uranium nitrate. The quantity of the latter, then, to be dissolved in 1000 c.c.

of water will be found from the equation, 636 : 71 : : x : 5; and x = 44.78.

44.78 grams of uranium nitrate are weighed off and dissolved in about 900 c.c. of water, the solution being purposely made too strong for reasons pointed out in the section on Chlorides. In order to bring this solution to its proper strength it is necessary to titrate with the uranium solution a solution of disodium phosphate[1] of such strength that each 50 c.c. shall contain 0.1 gram of P_2O_5, or 1000 c.c. 2 grams. The molecular weight of $Na_2HPO_4 + 12H_2O$ being 358, this amount of disodium phosphate in grams is equivalent to 142 grams of P_2O_5; the quantity of P_2O_5 corresponding to 2 grams, in terms of $Na_2HPO_4 + 12H_2O$, is found from the equation, 358 : 142 : : x : 2; and x = 5.042. This amount of pure, dry, and non-deliquescent Na_2HPO_4 is dissolved in 1000 c.c. of distilled water. If non-deliquescent disodium phosphate is not at hand, about 6 or 7 grams of the salt are dissolved in 1000 c.c. of distilled water; of this solution 50 c.c. are evaporated in a weighed platinum dish, and the residue gently heated, the disodium phosphate being thereby transformed into sodium pyrophosphate, $Na_4P_2O_7$. The molecular weight of $Na_4P_2O_7$ being 266, this corresponds to 142 grams of P_2O_5. If the solution is of the correct strength—*i. e.*, containing 0.1 gram of P_2O_5 in 50 c.c. of water—the residue should weigh 0.1873 gram, as is seen from the equation, 142 : 266 : : 0.1 : x; and x = 0.1873. Supposing, however, that the residue weighs 0.1921 gram, it is manifest that the solution is too strong and must be diluted, the degree of dilution being ascertained according to the equation, 0.1873 : 1000 : : 0.1921 : x; and x = 1025; *i. e.*, 1000 c.c. of the solution must be diluted to 1025 c.c. to make it of the proper strength.

In the case given, 50 c.c. were used; the 950 c.c. are then diluted with the amount of water found from the equation, 1000 : 1025 : : 950: x; and x = 973.75. Having thus obtained a solution of disodium phosphate of such strength that each 50 c.c. shall contain 0.1 gram of P_2O_5, this is titrated with the uranium solution, which has been made too strong in order to determine the amount of water that must be added to the latter. To this end a burette is filled with the uranium solution; 50 c.c. of the disodium phosphate solution are treated with a few drops of the tincture of cochineal and 5 c.c. of the acetic acid mixture (see below). This mixture is heated in a beaker and, as soon as the boiling point has been reached, titrated with the uranium solution until a trace of greenish color is noticed in the precipitate, which does not disappear on stirring. This point having been accurately determined by means of a second titration, the number of cubic centimeters of distilled water with

[1] A solution of chemically pure crystallized monopotassium phosphate can also be used for standardization (Sutton's Volumetric Analysis, 8th ed., p. 316).

which the remaining solution must be diluted is determined according to the formula: $C = \frac{N \cdot d}{n}$, in which C represents the number of cubic centimeters which must be added, N the number of cubic centimeters remaining after the test titration, n the number of cubic centimeters consumed in one titration to bring about the end reaction, and d the difference between the number of cubic centimeters used in one titration and that theoretically required.

The amount of distilled water necessary for dilution is now added and the solution again tested, when 20 c.c. will correspond to 0.1 gram of P_2O_5.

2. The acetic acid mixture is prepared by dissolving 100 grams of sodium acetate in a little water, adding 30 grams of glacial acetic acid and diluting the whole to 1000 c.c.

3. Tincture of cochineal. This may be prepared as follows: A few grams of cochineal granules are digested at ordinary temperatures with 250 c.c. of a mixture of 3 volumes of water and 1 volume of 94 per cent. alcohol. The solution is then decanted and ready for use. The residue may be utilized in the preparation of a fresh supply of the tincture.

Application to the Urine.—50 c.c. of clear filtered urine are treated with 5 c.c. of the acetic acid mixture, the object being to transform any monacid sodium phosphate present into diacid sodium phosphate, and to neutralize any nitric acid that may be formed during the titration, as otherwise the nitric acid would cause a partial solution of the precipitated uranyl phosphate. A few drops of the tincture of cochineal are added, when the mixture is heated to the boiling point and titrated as described above. Two titrations are usually required.

Should it be desired to use potassium ferrocyanide as an indicator, the uranium solution must have been standardized with the same indicator, as errors will otherwise arise. The technique is simple. A number of droplets of the potassium ferrocyanide solution (about 5 per cent.) are placed on a piece of white filter paper. After every addition of the uranium solution to the boiling urine a droplet of the mixture is placed upon the ferrocyanide stain. The end reaction is indicated by the occurrence of a brown color.

The results are calculated as follows: Supposing 15 c.c. of the uranium solution to have been used, the corresponding amount of P_2O_5 in 50 c.c. of urine is found from the equation, $20 : 0.1 :: 15 : x$; and $x = 0.075$. The percentage amount would, hence, be $0.075 \times 2 = 0.15$. Supposing the total amount of urine to have been 2000 c.c., the elimination of P_2O_5 would correspond to 3 grams.

The presence of sugar and albumin does not interfere with the method.

Removal of the Phosphates from the Urine.—Whenever it is necessary to remove the phosphates from the urine in the course of an analysis the urine is rendered alkaline by the addition of the hydrate of an alkaline earth and precipitated with a soluble calcium or barium salt. They may also be precipitated by means of neutral or basic lead acetate, in which case the excess of lead is removed by means of hydrogen sulphide or dilute sulphuric acid.

THE SULPHATES

The sulphuric acid found in the urine is derived essentially from the albuminous material which is constantly broken down in the body, a very small portion only of the inorganic sulphates being referable to the mineral constituents of the food. As was pointed out in the section on Reaction, sulphuric acid is constantly produced in the body, and, coming into contact with the so-called neutral phosphates present in almost all the tissues, transforms these into acid phosphates, both appearing in the urine. The alkaline carbonates, which are derived from the organic salts ingested by a process of oxidation, are also attacked by the sulphuric acid.

As the amount of food ingested is gradually diminished a point is reached when the body most tenaciously holds any alkaline salts that may still be present. A new source for the neutralization of acid is then found in the ammonia, which would otherwise have been eliminated as urea.

While the greater portion of the sulphuric acid excreted in the urine is found in the form of mineral sulphates, about one-tenth of the total amount may be shown to be in combination with aromatic substances belonging to the oxy-group; most important among these are the salts of phenol, indoxyl, and skatoxyl. Their amount increases and decreases with the degree of intestinal putrefaction, and hence serves as an index of its intensity.

The mineral sulphates have been termed preformed sulphates in contradistinction to the others, which are known as conjugate or ethereal sulphates. In the following pages the former will be designated by the letter A, the conjugate sulphates by the letter B, and the total sulphates as $A + B$.

The amount of $A + B$ excreted in the twenty-four hours by a normal individual varies between 2 and 3 grams, the ratio of A to B being as 10 to 1.

An increase in the elimination of the total sulphates is observed, as would be anticipated, in all cases in which an increased tissue destruction is taking place, as in acute febrile diseases. It must be remembered, however, that the quantity excreted is then not always greater than during convalescence, the diet remaining the

same. Here, as elsewhere in urinary studies, it is necessary to distinguish between a relative increase and an absolute decrease. In pneumonia and acute myelitis the highest figures have been observed, the increased elimination during the febrile period being especially marked.

	Fever diet.		Full diet.
	Fever.	No fever.	No fever.
Pneumonia	3.51 gm.	1.47 gm.	2.25 gm.
Acute myelitis	2.62 gm.	1.52 gm.	2.33 gm.

During convalescence the excretion of the sulphates is diminished, a retention analogous to that of the chlorides and phosphates taking place.

A considerable elimination of $A + B$ has also been observed in leukemia, in which an average of 2.46 grams is excreted, as compared with 1.51 grams by a healthy individual receiving the same amount and kind of food. In one case of acute leukemia 5.8 grams were eliminated on the day preceding death.

In diabetes mellitus, diabetes insipidus, esophageal carcinoma, progressive muscular atrophy, pseudohypertrophic paralysis, and eczema an increased elimination has likewise been observed, while in chronic renal disease a diminished excretion is the rule.

A study of the elimination of the *conjugate sulphates* and of the relation existing between A and B in disease is still more important than that of the total sulphates; but in both cases the data available are scanty, and further observations are urgently needed. v. Noorden regards the elimination of more than 0.3 gram of conjugate sulphates in the twenty-four hours as excessive, the patient being on an ordinary mixed diet.

The conjugate sulphates, as would be expected, are increased in all cases of increased intestinal putrefaction. In coprostasis the result of carcinoma the ratio of the preformed to the conjugate sulphates, normally 10, may diminish enormously. In one case, reported by Kast and Baas, it fell to 2, but rose to 7 and 8, and finally to 9.5 and 15 after an artificial anus had been established. I have observed a drop to 1.5 in a case of volvulus of ten days' standing. H. Baldwin notes a case of pernicious vomiting of pregnancy in which the factor $A : B$ was 1.9; following abortion it rose to 4 and a little later to 5.4. Biernacki found an increase in the elimination of conjugate sulphates amounting to from 0.15 to 0.5 gram pro die in cases of chronic parenchymatous nephritis, going hand in hand apparently with a decrease in the secretion of hydrochloric acid by the stomach; the normal amount, according to his observations, varies from 0.1973 to 0.2227 gram. In one case B fell from 0.4382 to 0.1505 during the administration of hydrochloric acid, to increase again to 0.4127 upon its discontinuance.

In accord with these observations are those of Wasbutzki and

Kast. The former found an increased elimination of *B* in cases of intense bacterial fermentation taking place in the stomach, while hydrochloric acid was either totally absent or present in greatly diminished amount. A diminished elimination was observed in cases of intense torular fermentation, hyperchlorhydria existing at the same time. In the absence of hydrochloric acid a normal or even a slightly diminished amount was observed in cases of intense acid fermentation, lactic acid and butyric acid being present in large quantities.

By neutralizing the gastric juice with large doses of sodium bicarbonate Kast was able to bring about a marked increase in the elimination of *B*, the ratio $A : B$ having fallen from 10.3 to 16.1 to 2.9 to 6.1. Personal observations have led me to the same conclusion. (See also section on the Aromatic Bodies.)

In obstructive jaundice the excretion of *B* is likewise increased; it returns to the normal as soon as the permeability of the biliary passages has again become established. The total sulphates were found diminished in cases of non-obstructive jaundice. In Böhm's cases of catarrhal jaundice the excretion of conjugate sulphates varied between 0.4 and 0.7 gram. Of interest in this connection are the observations of Müller, who notes the elimination of 0.29, 0.24, and 0.28 gram of conjugate sulphates on three consecutive days in a case of total obstruction of the biliary duct in consequence of a stone. The patient during this period was on a milk diet, and there can be little doubt that the low values are here referable to the pure lactic acid producing organisms crowding out the colon bacilli. On a meat diet the same patient passed 0.48 and 0.51 gram.

Other observers have obtained less constant results in their cases of catarrhal jaundice. In cases of hepatic cirrhosis and malignant disease of the liver, Eiger and Hopadze found increased amounts of conjugate sulphates.

In cases of diarrhea $A + B$, as well as *B*, is diminished, while $A : B$ is increased.

Quantitative Estimation of the Sulphates.—The principle of the method depends upon the fact that the mineral sulphates form an insoluble precipitate of barium sulphate directly when treated with barium chloride, while the conjugate sulphates do so only upon decomposition with strong hydrochloric acid under the application of heat. In order to estimate the mineral and conjugate sulphates, it is best to determine the total sulphates in one portion and the conjugate sulphates in another, the difference between the two giving the mineral sulphates.

Quantitative Estimation of the Total Sulphates (*Folin*).—50 c.c. of clear, filtered urine are treated with 5 c.c. of concentrated hydrochloric acid and 5 c.c. of a 4 per cent. solution of potassium chlorate. The mixture is boiled until it is colorless (five to ten minutes) and

then treated, while still boiling, with 25 c.c. of a 10 per cent. solution of barium chloride, *drop by drop*. It is kept on a hot-water bath or on an asbestos plate hot (but not boiling) for one-half to one hour. The precipitate is now collected on a Schleicher and Schüll filter, the weight of the ash of which is known (No. 589). Care should be taken never to allow the filter to run dry, and small amounts of hot water must be added to the last cubic centimeters remaining, the final traces being placed upon the filter with the aid of a rubber-tipped glass rod. The precipitate is washed with hot water for a half-hour, and at intervals of a few minutes hot ammonium chloride solution (5 per cent.) is substituted for the water, so that in all five or six additions of ammonium chloride take place in the course of the first twenty-minutes' washing. In the end a specimen of the washings must no longer be rendered cloudy, even on standing a few minutes, upon adding a drop of dilute sulphuric acid.

The paper filter is partially dried by folding and pressing gently between filter paper. It is then placed in a weighed crucible, covered with 3 to 4 c.c. of alcohol, and the alcohol ignited. The ash is heated, at first moderately, and almost completely covered with the lid, then only half covered, for five to seven minutes, until the contents of the crucible are white. The crucible, when cooled, is placed in a desiccator and weighed, the difference between the first and the second weighing giving the weight of the barium sulphate obtained from 50 c.c. of urine.

Quantitative Estimation of the Conjugate Sulphates (*Folin*).—200 c.c. of urine (diluted to a liter if necessary) are treated with 100 c.c. of a 10 per cent. solution of barium chloride, at ordinary temperature. The mixture is set aside for twenty-four hours and the clear supernatant fluid poured into a dry beaker by decanting. This preliminary decantation is necessary, as the barium sulphate precipitate will otherwise go through the paper. The decanted liquid is filtered, 150 c.c. of the clear filtrate, representing 100 c.c. of urine, measured into an Erlenmeyer flask, treated with 10 to 15 c.c. of concentrated hydrochloric acid and 10 to 15 c.c. of a 4 per cent. solution of potassium chlorate. The mixture is then heated to boiling and kept upon a boiling water bath until the barium sulphate has settled and the supernatant fluid is clear. The precipitate is filtered off, washed, dried, and weighed, as described above. The weight thus obtained, deducted from the amount found according to the first method, indicates the amount referable to the mineral sulphates. The molecular weight of $BaSO_4$ being 232.82, that of SO_3, 79.86, of H_2SO_4, 97.82, and of S, 32, the figure expressing the amount of H_2SO_4, SO_3, or S, corresponding to 1 gram of $BaSO_4$, is found according to the following equations:

$232.82 : 79.86 : : 1 : x$; and $x = 0.34301$; $\therefore$ 1 gram of $BaSO_4 =$ 0.34301 gram of SO_3.

232.82 : 97.82 : : 1 : x; and x = 0.42015; ∴ 1 gram of $BaSO_4$ = 0.42015 gram of H_2SO_4.

232.82 : 32 : : 1 : x; and x = 0.13744; ∴ 1 gram of $BaSO_4$ = 0.13744 gram of S.

To calculate results, it is only necessary to multiply the weight of the $BaSO_4$ by 0.34301, 0.42015, or 0.13744, in order to ascertain the amount of sulphuric acid contained in 50 c.c. of urine, in terms of SO_3, H_2SO_4, or S, respectively.

NEUTRAL SULPHUR

While the greater portion of the sulphur of the body is eliminated in an oxidized form, small amounts of non-oxidized sulphur bodies are likewise found in every urine. They are collectively spoken of as the neutral sulphur of the urine, and under normal conditions constitute from 12 to 15 per cent. of the total sulphur. The relation existing between the oxidized and the neutral form is, however, inconstant, and varies with the character of the diet, the degree of the protein metabolism, etc.

Of the nature of the neutral sulphur bodies which occur in *normal* urine, comparatively little is known. At the present time we are acquainted with only two substances belonging to this order, viz., certain sulphocyanides and cysteïn, or a body which is closely related to it. The greater portion of *the sulphocyanides* is undoubtedly derived from the saliva that has been swallowed and absorbed, while a smaller amount may be referable to the trace which is said to be present in normal, uncontamniated gastric juice. The amount of sulphur which is present in this form represents about one-third of the total quantity of the neutral sulphur. *Cysteïn* probably is an intermediary product of the normal metabolism of protein material. Under normal conditions, however, the greater portion is oxidized to sulphuric acid, and traces only escape to be eliminated as such.

Whether or not *taurocarbaminic acid,* which is a derivative of taurin, is a constant constituent of the urine remains an open question, but is very probable. We know, as a matter of fact, that the amount of neutral sulphur undergoes a distinct diminution in animals when the bile is prevented from entering the intestinal canal by establishing an external fistula. Under pathological conditions a corresponding increase is observed in cases of biliary obstruction, and the amount of neutral sulphur may then reach 40 per cent. of the total sulphur.

Thiosulphates, which are normally present in the urine of dogs and cats, do not occur in human urine under normal conditions. That they may be present in disease has been shown by Strümpell,

who found them in a case of typhoid fever. Further observations, however, are wanting.

Another sulphur body belonging to this class, which Abel discovered in the urine of dogs, and which appears to be identical with *ethyl sulphide*, has not been found in the urine of man.

The greatest increase in the amount of the neutral sulphur is observed under certain conditions associated with the appearance of *cystin*. Normally this is not present in the urine, while traces of *cysteïn*, or a closely related substance, as I have already stated, are found. According to Baumann and v. Udranszky, its appearance in the urine is closely connected with the formation of certain diamins, viz., cadaverin, putrescin, and a third diamin which is probably identical with saprin or neuridin. As these diamins were hitherto supposed to result only from the action of certain specific bacteria upon albuminous material, cystinuria was regarded as evidence of a definite infectious process. It is to be noted, however, that cystin itself does not occur in the feces, and that diaminuria does not necessarily accompany the cystinuria. As the result of personal observations I have been led to the conclusion that a causal connection does not exist between the two conditions, and that the diamins in question can be produced in the body tissues directly without the intervention of microörganisms. I regard cystinuria essentially as a metabolic anomaly, the result of a specific insufficiency on the part of certain tissues (liver) of the body. The condition may be temporary, but, as a rule, it is permanent. It may occur among several members of the same family, but it is noteworthy that no case has been reported in which a parent and child were cystinuric. Consanguinity among parents, which is not infrequently observed in cases of alkaptonuria, is the exception in cystinuria.

The amount of neutral sulphur which may be met with in cystinuria is subject to wide variation, but not infrequently exceeds 30 per cent. of the total sulphur. As a general rule, the amount of cystin eliminated in the twenty-four hours is less than 0.5 gram. At times, however, larger quantities are found, and on one occasion I obtained more than 1 gram. Clinically it is of interest in so far as its continued production may give rise to the formation of calculi.

Unless cystin occurs as a deposit, its presence will scarcely be suspected. The substance, however, may occur also in solution, and it not infrequently happens that attention is first drawn toward its existence in this state owing to the marked odor of hydrogen sulphide which such urines develop on standing. (See Hydrothionuria.) If acetic acid is then added in excess, the characteristic hexagonal plates may crystallize out. The same result is obtained by allowing the urine to undergo ammoniacal decomposition, as cystin is insoluble in solutions of ammonium carbonate.

Cystin crystallizes in hexagonal plates which are quite character-

istic, and not likely to be confounded with other crystalline elements that may be present in urinary sediments. If doubt should arise, their solubility in ammonia and hydrochloric acid, and their insolubility in acetic acid, water, alcohol, and ether, will lead to their identification.

The quantitative estimation of cystin is rather unsatisfactory, as no method is known which yields reliable results. On the whole, it is perhaps best to determine the neutral sulphur, and to refer the increase beyond its normal value to the presence of cystin.

Quantitative Estimation of the Neutral Sulphur in the Urine.—In one portion of the urine the oxidized sulphur, viz., the mineral and the conjugate sulphates, are estimated as described. In a second portion the total sulphur is determined, the difference indicating the amount of the neutral sulphur.

To determine the total amount of sulphur the following method is most conveniently employed:

Method of Höhnel-Glaser (*modified by Modrakowsky*).—1 or 2 grams of sodium peroxide are placed in a nickel dish, and covered with 50 c.c. of urine, added drop by drop. The fluid is evaporated to a syrup on a water bath, and further treated with 2 or 3 grams of the peroxide, which is added slowly while stirring. As soon as the reaction, which at first is quite vigorous, has subsided somewhat, the dish is removed from the water bath and heated over a *small* flame. If necessary, 1 to 3 grams more of the peroxide are added. The mass now forms brown drops and finally becomes thick; this ends the reaction. On cooling, the fusion is dissolved in hot water; the solution is filtered and feebly acidified with hydrochloric acid. Barium chloride is then added and the process continued as above described (Estimation of Sulphates).

UREA

Urea is the most important end product of the exogenous nitrogenous katabolism, and normally represents from 85 to 86 per cent. of the total amount of nitrogen that is eliminated through the kidneys. What proportion of the entire quantity is referable to the endogenous katabolism is as yet an open question, but it is unquestionable that by far the greater amount represents the excess of nitrogen which has been ingested and which is almost immediately eliminated. The actual quantity accordingly depends primarily upon the amount of nitrogenous food ingested, and varies more or less in different people and in different races. In most text-books the statement is found that the normal daily elimination of urea varies between 30 and 35 grams. This would imply that a smaller amount could be viewed as an abnormality, a conclusion to which I per-

sonally cannot subscribe, as there are many people in perfect health who never pass more than 20 to 25 grams of urea, because they ingest a corresponding quantity of nitrogenous foodstuffs. Unless the nitrogen content of the food ingested is known it would be unwarrantable to speak of lower or higher values as abnormal. In diabetics, for instance, an elimination of 100 to 150 grams can scarcely be viewed as evidence of a disturbed nitrogenous metabolism, if it is borne in mind that such patients generally consume a much larger quantity of nitrogenous foodstuffs as a part of their dietetic treatment.

The increased elimination of urea in febrile diseases, on the other hand, which may amount to 50 grams or more in the twenty-four hours, is generally ascribed to the greatly increased tissue destruction. The largest increase of this character is seen in those diseases which end by crisis and notably in pneumonia, where it may continue for two or three days after the crisis, and is then no doubt due to the resorption of the exudate.

Curious irregularities in the elimination of urea have been observed in cases of pernicious anemia, where periods of markedly increased albuminous disintegration alternate sometimes with such of nitrogenous retention.

An unusually large output of nitrogen and greatly in excess of the amount ingested is apparently a common feature of acute leukemia. Ebstein records a case in which 62 grams of urea were eliminated in twenty-four hours, and Edsall mentions an instance in which, with an intake of only 7.25 grams of nitrogen, 29.534 grams appeared in the urine.

In this connection it is interesting to note that an astonishing increase of the urinary nitrogen occurs on *x*-ray treatment in those cases of chronic leukemia in which a characteristic response so far as the effect upon the spleen and the number of the leukocytes is concerned, takes place, while in the negative cases this is not observed.

In purpura hæmorrhagica a notable increase of the urinary nitrogen occurs, apparently without relation to the hemorrhages. Edsall mentions an instance in which the patient, while ingesting not more than 3 or 4 grams, eliminated amounts varying between 14 and 23 grams.

A moderate increase has been found in severe cases of chronic leukemia, scurvy, minor chorea, and paralysis agitans. Observations made in cases of hystero-epilepsy have given rise to conflicting results. It is claimed, on the one hand, that the excretion of urea is diminished following convulsive seizures of a hystero-epileptic nature, in contradistinction to an increased elimination following true epileptic attacks.

Sine qua non for a normal or increased elimination of urea is, of course, a functional sufficiency of the liver, without which the formation of urea is more or less extensively impaired. In acute yellow

atrophy and also in Weyl's disease, notwithstanding the frequently not inconsiderable degree of fever, urea may thus disappear from the urine altogether. In cirrhosis a diminution is commonly observed, and may be further increased by the accompanying hyperemia of the portal system and the occurrence of ascites.

The third factor which regulates the output of urea is the condition of the kidneys. Whenever there is disease affecting that portion of the renal parenchyma which is concerned especially in the elimination of urea a diminished amount will be met with. However, as v. Noorden and others have pointed out, there are periods in the course of a nephritis when the urea output is quite normal.

Whenever any notable diminution in the excretion of urea is observed which cannot be accounted for by a correspondingly diminished ingestion of nitrogen, a careful study of the ammonia output is indicated. The two examinations supplement one another and combinedly furnish an excellent insight into the nitrogenous metabolism.

Quantitative Estimation of Urea.—Hypobromite Method.—The method most commonly used in the clinical laboratory is the one based upon the decomposition of urea into carbon dioxide and nitrogen in the presence of sodium hypobromite. The carbon dioxide thus formed is absorbed by an excess of sodium hydrate in the hypobromite solution, while the nitrogen is set free, and can be collected and measured; the determination of the corresponding amount of urea is then a simple matter.

The hypobromite solution is prepared from two stock solutions. The first of these contains 125 grams of bromine and 125 grams of sodium bromide in 1000 c.c. of water. The second is a 22.5 per cent. solution of sodium hydrate. Immediately before use equal portions of the two solutions are mixed and diluted with one and one-half volumes of water.

The reaction which takes place may be represented by the equation:

$$2NaOH + 2Br = NaBr + NaOBr + H_2O.$$

Various forms of apparatus, termed *ureometers,* have been suggested for the estimation of urea by this method.

The one most commonly in use is that of Doremus, and for most purposes this is entirely sufficient. When accurate metabolic studies are to be carried on, however, more exact methods are necessary, and for such purposes, Folin's method may be employed (see below).

Doremus' Method.—The general construction of the instrument is seen in Fig. 148. A small amount of urine is poured into *B* while the stopcock (*C*) is closed. This is then opened for a moment and again closed, so as to fill its lumen. The tube *A* is washed out with water and filled with the hypobromite solution. The tube *B* is filled with urine to the zero mark, and 1 c.c. (or less, if the urine is

concentrated) is allowed to mix with the hypobromite solution (see above) in *A*. After all bubbles of gas have disappeared the reading is taken. Each small division corresponds to 0.001 gram of urea and every ten divisions hence to 0.01 gram, for the amount of urine used.

The urine must be free from albumin and should not contain more than 1 per cent. of urea. If necessary it is diluted with water.

In the presence of ammonium compounds the results may be faulty, and in cases where this is suspected it is advisable to resort to more accurate methods, such as that of Folin.

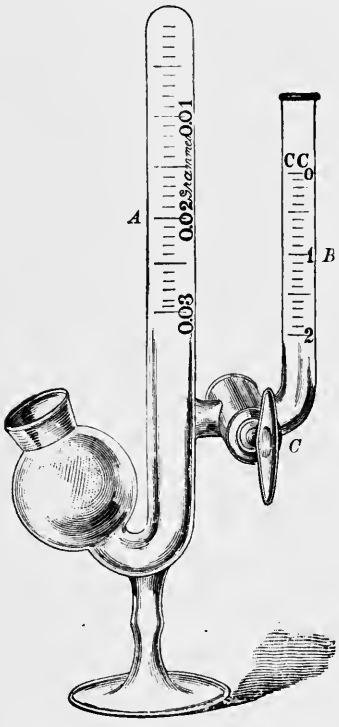


Fig. 148.—Doremus-Heinz ureometer.

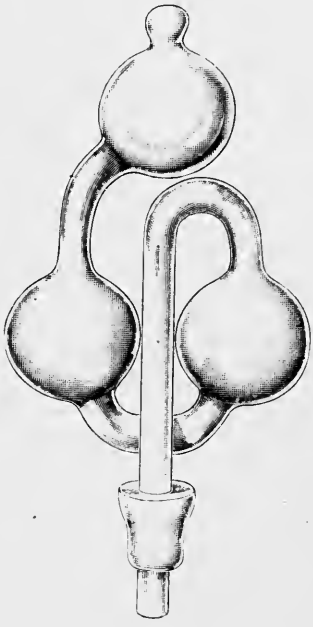


Fig. 149.—Folin's safety tube.

Method of Folin.—This is based upon the following considerations: At a temperature of about 153° to 160° C. crystallized potassium acetate boils in its water of crystallization. In such a solution urea is quantitatively decomposed into ammonia and carbon dioxide. The ammonia can then be driven off after rendering the mixture alkaline, and be estimated either colorimetrically, after Nesslerizing, or by titration in the usual manner. The corresponding amount of urea is ascertained by calculation. At the same time, however,

the preformed ammonia is obtained, and it is hence necessary to eliminate this source of error by a separate estimation of this form. This is conveniently done according to a method which has likewise been suggested by Folin (see below).

Method.—The urine is diluted so that 1 c.c. contains 0.75 to 1.5 mg. of urea nitrogen. Dilutions of 1 in 20, 1 in 10, or rarely 1 in 5 are usually adequate. One c.c. of the diluted urine, measured with an Ostwald pipette, is placed in a large Jena test-tube (20 by 200 mm.) which has been previously charged with 7 gms. of dry potassium acetate (*free from lumps*), 1 c.c. of 50 per cent. acetic acid, and a small quartz pebble or a little powdered zinc (not zinc dust) to prevent bumping. A temperature indicator containing powdered chloride-iodide of mercury, which melts at 155° C., and solidifies again at 148° C., is likewise introduced into the tube (Eimer and Amend). This is then closed with a rubber stopper, carrying an empty narrow "calcium chloride tube" (without bulb) (25 by 1.5 cm.) as a condenser, the whole apparatus being suspended over a micro-burner. In about two minutes the mixture begins to boil and the indicator melts. The boiling is continued for ten minutes, in a gentle, even manner, by which time the decomposition of the urea is complete. The contents of the tube are then diluted with 5 c.c. of water, introduced through the calcium chloride tube, when an excess of a saturated aqueous solution of caustic soda or potassium carbonate (2 c.c.) is added and the liberated ammonia driven off by means of a strong air current (see estimation of ammonia) and received in a 100 c.c. measuring flask, containing about 35 c.c. of water and 2 c.c. of $\frac{n}{10}$ acid. A suction of ten minutes is usually sufficient, if a good pump and a pressure of 40 to 45 pounds is available. The amount of ammonia is then estimated colorimetrically after Nesslerizing, as described in the section on Total Nitrogen Estimation.

The potassium acetate which is used in this method, need not be dried if of a German brand, while the American products contain too much water and must be dried before use. This is conveniently done by placing about a pound at a time in a large porcelain plate and allowing it to stand on a warm plate (115° C.) for twenty-four hours.

During the process of boiling, Folin recommends that the flame of the micro-burner do not exceed 0.5 cm. and that the bottom of the test-tube be some distance above it. If too much heat is applied the acetate cakes at the bottom of the tube, if too little it cakes at the top.

If a colorimeter is not available the determination of the ammonia can also be carried out by titration as described in the section on Total Nitrogen Estimation.

In the presence of sugar Folin's method, as described above, is modified as follows: 1 c.c. of urine which has been previously diluted

from 20 to 100 times (so that 1 c.c. contains about 0.1 mg. of urea nitrogen) is decomposed with acetate as described above. The ammonia is collected in a second test-tube, containing about 2 c.c. of water and 0.5 c.c. of $\frac{n}{10}$ hydrochloric acid, when a couple of c.c. of water and 3 c.c. of diluted (1.5) Nessler solution are added. The resultant colored solution is then washed into a 10 c.c. measuring flask and the volume made up to 10 c.c. The whole is transferred to a dry cylinder of a Duboscq colorimeter and the depth of color determined in the usual way against 1 mg. of nitrogen per 100 c.c. of solution (see also Total Nitrogen Estimation—Folin's method).

NITROGEN

Estimation of Nitrogen.—For the purpose of estimating the total amount of nitrogen in the urine, the method of Kjeldahl is most conveniently employed.

Kjeldahl's Method.—*Principle.*—The organic matter of the urine is decomposed by means of sulphuric acid, when all the nitrogen which is not present in combination with oxygen is transformed into ammonia. After adding sodium hydrate in excess the ammonia is distilled off and received in a known quantity of titrated acid, the excess being retitrated with sodium hydrate. In this manner the amount of ammonia and the corresponding quantity of nitrogen are ascertained, it being remembered that 17 grams of ammonia correspond to 14 grams of nitrogen.

Reagents Required.—1. Gunning's mixture. This consists of 15 c.c. of concentrated sulphuric acid, 10 grams of potassium sulphate, and 0.5 gram of cupric sulphate. In the place of Gunning's mixture one of 500 c.c. of concentrated sulphuric acid and 100 grams of phosphoric anhydride may also be employed, and has the advantage that oxidation proceeds more rapidly.

2. A solution of sodium hydrate containing 270 grams in the liter (sp. gr. 1.243).

3. Pulverized talcum or granulated zinc.

4. A one-fourth normal solution of sulphuric acid.

5. A one-fourth normal solution of sodium hydrate.

Apparatus Required (Fig. 150).—This consists of a retort of about 750 c.c. capacity (*A*), which is connected with a Kjeldahl distilling tube (*B*), and through this with a Städeler condenser (*C*). The ammonia is received in the nitrogen bulb at *D*. In addition a Kjeldahl digesting flask of 200 to 300 c.c. capacity is required.

Method.—5 or 10 c.c. of urine are placed in the digesting flask and treated with Gunning's mixture. To this end it is best to add the sulphuric acid and cupric sulphate first, to heat until sulphuric acid vapors are given off in abundance, and then to add the potas-

sium sulphate. The heating is continued until the solution becomes entirely clear and almost colorless, the flask being inclined at an angle of about 45 degrees. *Vigorous ebullition should be avoided.* If the sulphuric acid-phosphoric anhydride mixture is to be employed, the urine is first treated with 0.4 gram of mercuric oxide, when 10 c.c. of the acid mixture are added. Digestion is then carried on as described. Toward the end of digestion, in either case, it is advantageous to throw a few crystals of potassium permanganate into the fusion, so as to insure complete oxidation.

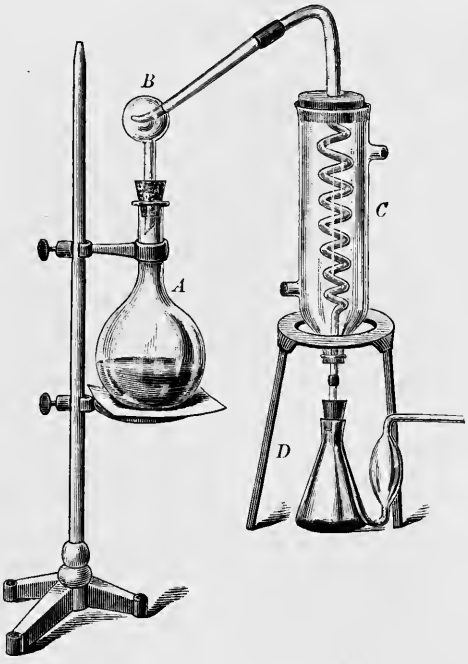


FIG. 150.—Kjeldahl's nitrogen apparatus.

Upon cooling, the contents of the flask are transferred to the retort with the aid of a little water, and slowly treated with a moderate excess of the sodium hydrate solution. As a general rule, 40 c.c. for each 5 c.c. of sulphuric acid are sufficient. A little pulverized talcum or a few pieces of granulated zinc are finally added; the retort is connected with the condenser with the interposition of the distilling tube and the distillation begun. The talcum or zinc serves the purpose of preventing undue frothing and bumping. The distillation is continued until about two-thirds of the solution have passed over. The distillate is received in the nitrogen bulb, which should contain a carefully measured quantity of the one-fourth normal solution of sulphuric acid. As a general rule, 50 c.c. are suffi-

cient. As soon as the distillation is completed the condenser is disconnected, washed out with a small amount of distilled water, and the washings added to the distillate. After the addition of a few drops of tincture of cochineal or dimethyl-amino-azo-benzol the excess of sulphuric acid is retitrated with the one-fourth normal solution of sodium hydrate, and the amount found deducted from the 30 c.c. used. The titration should be continued until every trace of yellow (in the case of the cochineal) has disappeared and a pure rose color is obtained, or, in the case of the dimethyl-amino-azo-enzol, until the last trace of red has disappeared and the solution has turned yellow. The difference multiplied by 0.0035 will indicate the amount of nitrogen present in the 5 or 10 c.c. of urine. The corresponding amount of urea is found by multiplying this figure by 20.

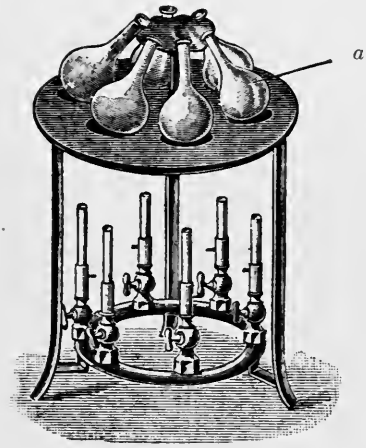


FIG. 151.—Kjeldahl's apparatus for the simultaneous oxidation of six specimens: *a*, Kjeldahl flasks.

Whenever several nitrogen determinations are to be carried out daily it is convenient to make use of a special apparatus, which permits of such determinations being conducted at one time. The general plan of the outfit is seen in the accompanying illustrations (Figs. 151 and 152).

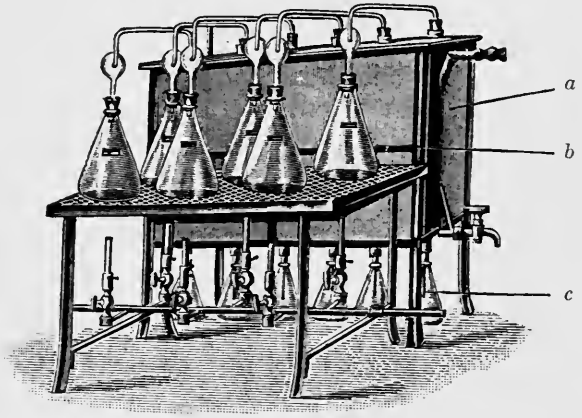


FIG. 152.—Kjeldahl's apparatus for the simultaneous distillation of six specimens. *a*, condenser; *b*, distillation flasks; *c*, receivers.

Instead of *distilling* off the ammonia, the suction arrangement, illustrated in Fig. 153 may be employed. To this end the digesting flask *B*, with the dissolved fusion, is connected up on the one hand with a cylinder, *A*, containing the necessary amount of alkali, and on the other with the cylinder *C*, containing the fourth normal sulphuric acid, and with which a Folin absorption tube *D* dips. Cylinder *C* is connected with a suction pump, which is then allowed to carry a current of air through the system of cylinders until all the ammonia has been exhausted from *B*.[1] The time for this will vary with the water pressure and must be ascertained for every laboratory.

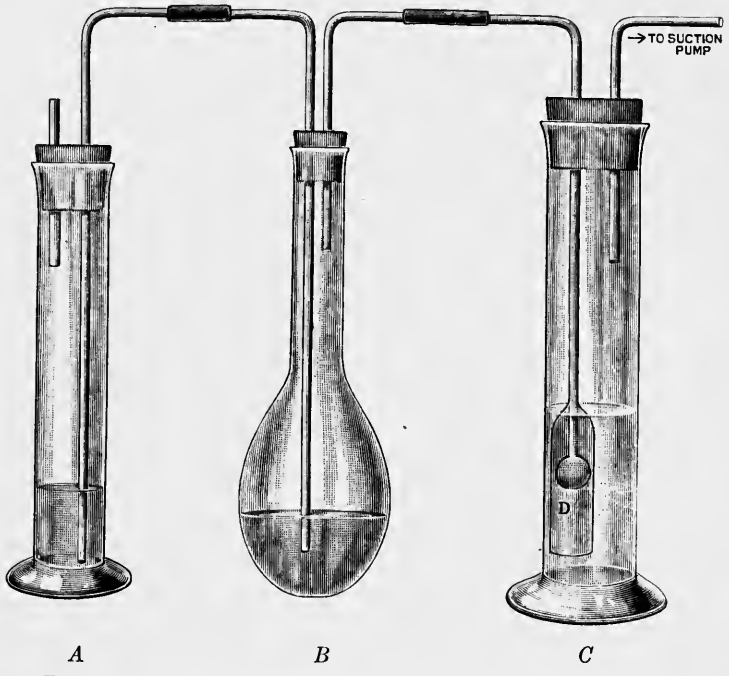


FIG 153.—Apparatus for the estimation of the total nitrogen as ammonia.

Folin and Farmer's Method.—This modification of the original Kjeldahl method will be found especially serviceable in the smaller hospital laboratories.

Five c.c. of urine are diluted in a measuring flask to 50 c.c., if the specific gravity is over 1.018 or to 25 c.c. if it is below 1.018. One c.c. of the diluted urine, measured off with an Ostwald pipette, is then placed in a large test-tube of Jena glass (20 to 25 by 200 mm.), treated with 1 c.c. of concentrated sulphuric acid, 1 gram of potas-

[1] The immediate effect of turning on the suction will, of course, be the transfer of the alkali from *A* into *B*, which should be done slowly.

sium sulphate and 1 drop of a 5 per cent. copper sulphate solution, together with a small, clean quartz pebble (to prevent bumping). The mixture is boiled over a micro-burner (No. 2587 of Eimer and Amend) for about six minutes, *i. e.*, about two minutes after it has become colorless. After cooling for a few minutes until it begins to become viscous (but not yet solid) about 6 c.c. of water are added, at first a drop at a time, then more rapidly so as to prevent the

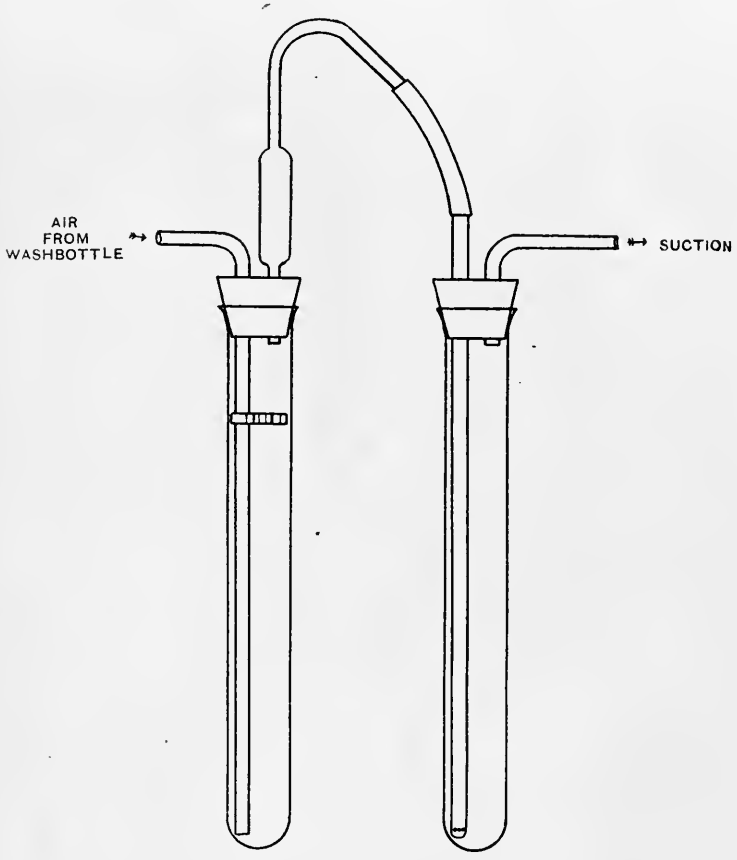


FIG. 154.—Apparatus for use with suction.

mixture from solidifying. An excess of sodium hydrate (3 c.c. of a saturated solution) is then added, when the liberated ammonia is aspirated into a 100 c.c. measuring flask, containing about 20 c.c. of water and 2 c.c. of $\frac{n}{10}$ hydrochloric acid. The air current should be moderate for the first two minutes, but subsequently, for about eight minutes vigorous suction is desirable. The contents of the receiving flask are then diluted to about 60 c.c. and 1 mg. of nitrogen

in the form of ammonium sulphate dissolved in approximately the same volume of water in a second flask. Both specimens are now Nesslerized by treating with 5 c.c. each of Nessler's reagent which *immediately* before has been diluted with 25 c.c. of water. Both flasks are finally diluted with distilled water to the 100 c.c. mark and the relative intensity of the colors determined by means of a suitable colorimeter (Duboscq). The reading of the standard divided by the reading of the unknown gives the nitrogen, in milligrams, in the volume of urine used. With the Duboscq apparatus the standard may be set at 20 mm.

The ammonium sulphate used for the final comparison must be free from pyridin bases. To this end a high-grade ammonium sulphate is decomposed with caustic soda and the ammonia gas passed into pure sulphuric acid by means of an air current, when the salt can be precipitated by alcohol and dried over sulphuric acid.

The Nessler reagent is prepared in the usual manner, mercuric iodide, however, being substituted for the chloride, as recommended by Winkler.

In order to prevent any of the alkali from getting into the receiving flask, while the air current is passing, it is recommended to cut a small rubber disk from a doubly perforated stopper and to adjust this in the tube as shown in the accompanying Fig. 154, a couple of notches being made laterally to allow for the return flow of any fluid that has passed beyond.

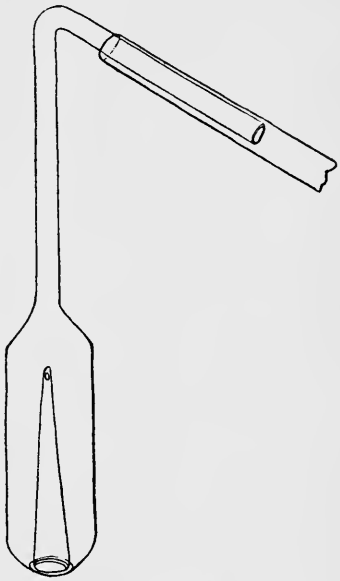


Fig. 155.—Improvised hood.

To secure the proper absorption of the ammonia in the receiving flask, it is advantageous to use a tube which is sealed at the bottom but provided with a few small holes which can be readily made by puncturing the heated end with a piece of platinum wire.

Instead of using the colorimetric method described above one can also titrate the ammonia evolved from so small a quantity of urine as 1 c.c. by receiving the gas in 10 c.c. of $\frac{n}{10}$ acid plus 40 c.c. of water, and then retitrating the excess with $\frac{n}{50}$ alkali using alizarin red as indicator.

In laboratories in which a hood is not available one can readily improvise one by constructing a simple device like that pictured in Fig. 155, as suggested by Folin. This is placed in the mouth of the

tube used for digestion and is connected with the suction pump with the interposition of a wash bottle containing 10 per cent. caustic soda solution.

AMMONIA

Every urine contains a small amount of ammonia, which normally varies but little, and corresponds to from 4.1 to 4.64 per cent. of the total amount of nitrogen, viz., to about 0.7 gram in the twenty-four hours. It is present in combination with various acids of the urine, and in all likelihood represents a small amount of the ammonia which has not been transformed into urea, but has been utilized to saturate the affinities of a slight excess of acid, formed during the nitrogenous metabolism of the body over the available fixed alkalies.

In man an increased elimination of ammonia is observed whenever an increased formation of acids occurs, or whenever a sufficient supply of oxygen is not available. In the latter case, no doubt, the increased elimination is owing to the fact that in consequence of the deficient supply of oxygen the synthetic formation of urea is impeded in the liver. As this organ, moreover, is the principal seat of the synthesis of urea, we can readily understand that extensive parenchymatous degeneration, as in acute yellow atrophy, in phosphorus poisoning, etc., will lead to an increased elimination of ammonia.

In any event, the relative increase of the ammonia is the essential factor, while variations in its absolute quantity are of secondary importance. Some of the results which have been obtained in various diseases are given in the following table:

	Per cent.
Normal values	4.10 to 4.64
Febrile diseases	5.72 to 6.70
Carcinoma of the liver	6.40 to 24.50
Liver abscess (actinomycosis)	10.60
Circulatory dyspnea	13.10 to 32.20
Respiratory dyspnea	6.60 to 14.30

Abnormally high absolute values are quite constantly observed in diabetes, in which a daily elimination of from 4 to 5 grams may be regarded as common. In a general way the amount of ammonia in cases of diabetes gives an idea of the amount of organic acids; but, as Herter has pointed out, we cannot detect moderate quantities of organic acids in this way. (See Oxybutyric Acid.)

In cases of pernicious vomiting of pregnancy Williams found a large increase of ammonia, up to 20 to 45 per cent., while this does not occur in nervous vomiting and in eclampsia. It is advised that in such cases the uterus be emptied, when the ammonia is said to drop at once.

A slight rise occurs also in normal pregnancy and reaches its maximum during labor.

Very curiously a diminished elimination of ammonia is observed in many cases of nephritis so long as symptoms of venous stasis do not exist.

In a case of pernicious anemia relative amounts, varying between 3.3 and 5.6 per cent., were obtained during the days immediately preceding death.

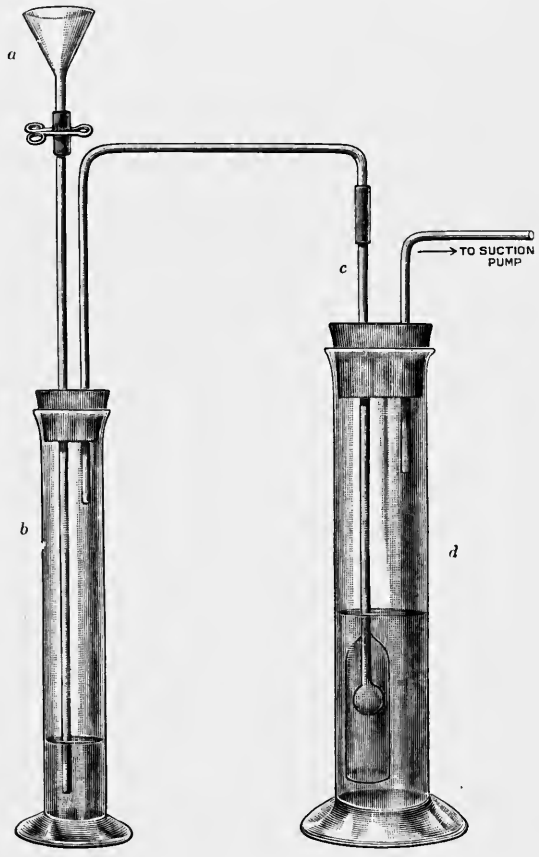


FIG. 156.—Apparatus for the estimation of ammonia or acetone.

Quantitative Estimation.—Folin's Method.—The most recent method suggested by Folin for the quantitative estimation of ammonia in the urine is the following:

From 1 to 5 c.c. of urine, measured off with an Ostwald pipette, are placed in a test-tube together with a few drops of a solution

containing 10 per cent. of potassium carbonate and 15 per cent. of potassium oxalate, besides a few drops of kerosene or heavy crude machine oil (to prevent foaming). The exact amount of urine depends upon its content in ammonia nitrogen, which should be between 0.75 and 1.5 mg. With normal urine 2 c.c. will usually be found sufficient while with very dilute urine 5 c.c. may be necessary, and with diabetic urine rich in ammonium salts 1 c.c. will be sufficient, or, indeed, it may be necessary to dilute the urine in such cases. A strong current of air is passed through the mixture, and the ammonia collected in a 100 c.c. measuring flask, containing about 20 c.c. of water and 2 c.c. of $\frac{n}{10}$ acid, when it is Nesslerized and the amount of ammonia determined as described on p. 361. Folin remarks that no absolutely sharp end-point is obtainable when a rapid air current is passed through the urine. A trace of something capable of giving a color with Nessler's solution continues to come off long after all the ammonia has been removed. What this substance is is not known; its effect in actual ammonia determinations, however, is so small as to be hardly, if at all, perceptible.

Folin has pointed out that a water-pressure of not more than 40 to 45 pounds is sufficient to obtain an air current of such strength that a ten minutes' suction will suffice to carry all the ammonia over. A good pump, however, is a prerequisite, and Folin recommends No. 2458 (list 120) listed by Kny-Scheerer & Co.

URIC ACID

The uric acid which is found in the urine is derived from two sources, viz., the nucleins and purin bases ingested (exogenous uric acid) and the nucleins of the body tissues (endogenous uric acid). Among the latter the leukocytes are the most important. Whether or not the substance may also be formed synthetically in the human being is not definitely known, but not impossible.

Under normal conditions the daily elimination varies between 0.2 and 1.5 grams, thus constituting $\frac{1}{20}$ to $\frac{1}{120}$ part of the total urinary nitrogen. The amount, as would be expected, normally depends primarily upon the character of the diet. According to Horbaczewski, there is an increased elimination of the substance five hours after the ingestion of a full meal, which is referred to the disappearance of the digestive hyperleukocytosis and the associated leukocytolysis.

Some observers have attached much importance to the relation existing between the elimination of uric acid and urea, and are inclined to assume the existence of a special *uric acid diathesis* when this relation continuously exceeds the usual standard of 1 to 50 or 1 to 60. This question is an extremely intricate one, and we are

scarcely in a position to speak definitely of the significance of such variations. On the one hand, there can be no doubt that an unusually high uric acid coefficient may be met with in individuals who are apparently in good health, while in others, in whom larger actual amounts of uric acid are eliminated than are usual, normal or even subnormal values may be found. The entire question of the uric acid diathesis is in a chaotic condition, and it would, perhaps, be well to speak of such a diathesis only when a distinct increase is *continuously* observed. That numerous symptoms of a neurasthenic type are often seen when the uric acid coefficient is increased is a matter of daily observation, but it would be premature to regard this symptom as a causative factor of the disease in question. Even in gout it can scarcely be said that uric acid has been proved the *materia peccans*, and our knowledge concerning the etiology of the disease is still as obscure as when Garrod showed that an accumulation of uric acid occurred in the blood of such patients. Hitherto it has been supposed that the deposition of urates in the joints and periosteum of gouty patients is referable to a diminished alkalinity of the blood, and that acute paroxysms result whenever an increase in its alkalinity occurs, leading to a resorption of the urates previously deposited and a consequent flooding of the system with the material in question. As a matter of fact, a considerable diminution in its excretion is observed immediately preceding an attack, while during the paroxysm and immediately following it a corresponding increase is noted. Numerous investigations, however, have shown that distinct changes in the alkalinity of the blood do not occur in gout, and that an increase in the amount of uric acid in the blood may not only be observed in this disease, but in other diseases as well which are not associated with gouty symptoms. The conclusion is hence justifiable that the presence of uric acid in the blood *per se* cannot be offered as an explanation of the occurrence of a gouty attack. Futcher, who has studied a number of cases of gout with modern methods, states that he almost invariably found that before the onset of the acute symptoms the uric acid was below and often far below 0.4 gram. On the second or third day after the beginning of acute symptoms the uric acid curve steadily rises, reaching 0.8 to 1.9 grams or even higher values. With the subsidence of the acute symptoms the curve gradually falls below the lower limit of the normal, and in the interval between the acute attacks the excretion may be only 0.1 to 0.2 gram daily. In one very marked chronic case Futcher found no uric acid excretion whatever on certain days during the interval. The phosphoric acid curve runs a course almost parallel to that of the uric acid, which suggests quite strongly that even in gout the uric acid is derived from nucleins, and is not formed synthetically, as might possibly be imagined.

The greatest increase in the elimination of uric acid is observed

in leukemia, in which the quantity may amount to over 12 grams in the twenty-four hours (case of Magnus-Levy). That the increased elimination in this disease is referable to the enormous increase in the number of leukocytes and consequent leukolysis can scarcely be doubted. In other diseases which are associated with a high grade of leukocytosis, and especially those in which the disease terminates by crisis or hastened lysis, such as erysipelas and pneumonia, a considerable increase is likewise observed, and is referable to the same cause. This increase is especially marked immediately after crisis has occurred, but it not infrequently precedes it by several hours. In the other febrile diseases an absolute increase is less marked and inconstant.

In diabetes a diminished amount of uric acid is usually found. Cases may be seen, however, in which, associated with a diminution or an entire disappearance of the sugar, a most marked increase occurs, amounting in some cases to 3 grams in the twenty-four hours. To this condition the term *diabetes alternans* has been applied.

In acute articular rheumatism an increased elimination is observed so long as the temperature remains high, while with approaching convalescence the amount returns to normal, and may even fall below normal. In chronic rheumatism, on the other hand, no constant relations have been observed.

In the ordinary forms of anemia and chlorosis the amount of uric acid is quite constantly diminished, as also in chronic interstitial nephritis, chronic lead poisoning, progressive muscular atrophy, and pseudohypertrophic paralysis.

According to Krainsky, Haig, and Caro, a decrease in the output of uric acid precedes the epileptic attack, and is subsequently followed by a rise to the same degree. Haig also noticed this in connection with attacks of migraine.

Rather low amounts are reported by Edsall in a case of purpura hæmorrhagica.

Of special interest is the observation by Edsall that in those cases of chronic leukemia in which there is a response to *x*-ray treatment uric acid and purin bases are at once markedly increased.

Quantitative Estimation of Uric Acid. — Folin's Modification of Hopkins' Method.—This is the most convenient method for the estimation of uric acid in the urine, and as accurate as the more complicated procedure of Ludwig-Salkowski. It is based upon the precipitation of uric acid by ammonium sulphate, as ammonium urate, the decomposition of the latter by sulphuric acid, and the estimation of the liberated uric acid by titration with potassium permanganate. To precipitate the uric acid, and also to remove the small amount of mucoid substance which is found in every urine, the following reagent is employed: 500 grams of ammonium sulphate and 5 grams of uranium acetate are dissolved in 650 c.c. of water, to which solu-

tion 60 c.c. of a 10 per cent. solution of acetic acid are further added. The resulting solution measures about 1000 c.c.; 75 c.c. of the reagent are added to 300 c.c. of urine in a flask holding 500 c.c. After standing for five minutes the mixture is filtered through two folded filters, and thus freed from the mucoid body, which is carried down with the uranium phosphate in acid solution. The filtrate is divided into two portions of 125 c.c. each, which are placed in beakers and treated with 5 c.c. of concentrated ammonia. After stirring a little the solutions are set aside until the next day. The supernatant fluid is then carefully poured off through a filter (Schleicher and Schüll, No. 597); the precipitated ammonium urate is collected with the aid of a small amount of a 10 per cent. solution of ammonium sulphate and washed with the same reagent. Traces of chlorides do not interfere with the subsequent titration, and the process of filtration and washing can be completed in from twenty to thirty minutes. The ammonium urate is washed into a beaker, after opening the filter, using about 100 c.c. of water; 15 c.c. of concentrated sulphuric acid are then added, and the solution is titrated at once with a one-twentieth normal solution of potassium permanganate. Toward the end of the titration Folin suggests to add the permanganate in portions of two drops at a time, until the *first* trace of a rose color is apparent throughout the entire fluid. Each cubic centimeter of the reagent corresponds to 0.00375 gram of uric acid. A final correction (of 0.003 gram for every 100 c.c. of urine employed) is necessary, owing to the slight extent to which ammonium urate is soluble.

Preparation of the One-twentieth Normal Solution of Potassium Permanganate.—As the molecular weight of potassium permanganate is 157.67, one would expect that a normal solution of the salt should contain this amount in grams dissolved in 1000 c.c. of water. But the substance generally acts in the presence of free acids, upon deoxidizing substances, by losing 5 atoms of oxygen of the 8 atoms contained in 2 molecules, as is seen in the following equation:

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 10CO_2 + 8H_2O.$$

It follows that two-fifths of the molecular weight, or 63.068 grams, are the equivalent of 1 oxygen atom. But as oxygen is diatomic and the volumetric normal is calculated for monatomic values, this number must be divided by 2, and 31.534 grams of potassium permanganate should therefore be present in 1 liter of normal solution. A one-tenth normal solution would hence contain 3.1534 grams, and a one-twentieth normal solution 1.576 grams pro liter. This amount is weighed off and dissolved in 950 c.c. of water, when the solution is brought to the proper degree of dilution by titration with a one-twentieth normal solution of oxalic acid. A one-twentieth normal solution of oxalic acid contains 3.142 grams of the acid in 1000

c.c. of water. One c.c. of the one-twentieth normal solution of potassium permanganate should correspond to 1 c.c. of the oxalic acid solution. The titration is best conducted by diluting 10 c.c. of the oxalic acid solution to 100 c.c. with distilled water and adding 15 c.c. of concentrated sulphuric acid, so as to bring the temperature of the liquid to from 55° to 65° C. The potassium permanganate solution is then added drop by drop until the red color no longer disappears on stirring, but persists for at least thirty seconds.

THE PURIN (XANTHIN) BASES

The purin bases which have been found in the urine are xanthin, hypoxanthin, heteroxanthin, paraxanthin, guanin, and adenin. Together with uric acid they are termed the alloxur or purin bodies. Unlike uric acid, they also occur as such in animal as well as vegetable tissues. The amount which appears in the urine under normal conditions is very small, constituting about 10 per cent. of the uric acid. Larger quantities may be met with in various diseases, and, generally speaking, an increase in the amount of uric acid is associated with an increase of the xanthin bases. This is, however, not invariably the case, and at times it may be observed that an increase of the uric acid is accompanied by a diminution of the xanthins, and *vice versa*. These varying relations can, of course, be readily understood if we remember that uric acid is an oxidation product of the xanthin bases, and that their ultimate origin is the same. The largest quantities of xanthin bases are found in leukemia; Magnus-Levy has reported a case with 0.321 gram.

Individually the xanthin bases are of little clinical interest Xanthin has once been found in a urinary sediment, and has in several instances been encountered as the principal constituent of vesical calculi. Its normal quantity is said to vary between 0.02 and 0.03 gram. Larger quantities are found after a meal rich in nucleins, in leukemia, nephritis, pneumonia, etc.

Paraxanthin and heteroxanthin are present only in traces, as is apparent from the fact that Krüger and Salomon were able to obtain but 7.5 grams of heteroxanthin from 10,000 liters of urine.

Quantitative Estimation.—Salkowski's Method.—600 c.c. of urine are precipitated with 200 c.c. of magnesia mixture (composed of 1 part of crystallized magnesium sulphate, 2 parts of ammonium chloride, 4 parts of ammonium hydrate, and 8 parts of distilled water), when a 3 per cent. ammoniacal solution of silver nitrate is added to from 700 to 750 c.c. of the filtrate. The proportion should be 6 c.c. for every 100 c.c. of urine. If the precipitated silver chloride formed in the beginning does not disappear on stirring, a little more ammonium hydrate is added. A flaky precipitate next separates out, and is allowed to settle. In order to test whether enough of the silver

nitrate solution has been added, a few cubic centimeters of the supernatant fluid are acidified with nitric acid. If a distinct cloudiness, referable to silver chloride, appears, enough has been added. Otherwise the few cubic centimeters that were employed for this test are rendered alkaline again with ammonia, poured back, and treated with more silver solution until the required amount has been reached. After standing for one hour the mixture is filtered and the precipitate washed with water until all the free silver has been removed. The filter is then perforated, the precipitate washed into a flask with from 600 to 800 c.c. of water, acidified with hydrochloric acid, and decomposed with hydrogen sulphide. The excess of hydrogen sulphide is removed by heating on a water bath, when the silver sulphide is filtered off and the filtrate evaporated to dryness. The residue is treated with from 25 to 30 c.c. of dilute sulphuric acid (1 to 100). This solution is brought to the boiling point and is allowed to stand over night. The uric acid which has separated out is filtered off, washed with a small amount of dilute sulphuric acid (not more than 50 c.c.), then with alcohol and ether, and weighed. To the resulting weight 0.0005 gram is added for every 10 c.c. of the acid filtrate, to allow for the trace of uric acid which is thus lost.

After having filtered off the uric acid the filtrate is again treated with ammonia and silver solution, and the xanthin bases thus precipitated. The precipitate is collected on a small filter, washed with water, dried, and incinerated. The ash is dissolved in nitric acid, and the silver estimated by titration with a solution of potassium sulphocyanide, using ammonioferric alum as an indicator. (See Chlorides.) The solution of potassium sulphocyanide employed in the estimation of the chlorides may be used, and is of such strength that 1 c.c. corresponds to 0.00734 gram of silver. As 1 atom of silver in a mixture of the silver compounds of guanin, xanthin, hypoxanthin, etc., represents 0.277 gram of nitrogen or 0.7381 gram of the alloxur bases, it is apparent that 1 c.c. of the potassium sulphocyanide solution will represent 0.002 gram of nitrogen and 0.00542 gram of alloxur bases. In every case an accurate record must, of course, be kept of the amount of urine and filtrate used.

The amount of alloxur bases found by Salkowski in the normal urine of twenty-four hours varied between 0.0286 and 0.0561 gram.

HIPPURIC ACID

Hippuric acid is a constant constituent of normal urine, 0.1 to 1 gram being excreted in the twenty-four hours. That it is derived, to some extent at least, from albuminous material is proved by the fact that its elimination is not suspended during starvation nor

during the administration of a purely albuminous diet. It is in part derived from the proteins of the body, as proved by the fact that its elimination does not cease during starvation. Another portion is possibly referable to the liberation of phenylpropionic acid during the process of intestinal putrefaction, and the consequent formation of benzoic acid in the blood and its union with glycocoll to form hippuric acid. A considerable portion, further, is derived from benzoic acid or its derivatives, which occur in many fruits, and are transformed into hippuric acid in the body. Among those which are particularly rich in these substances may be mentioned the red bilberry, prunes, coffee beans, green gages, etc., and in all cases in which an increased elimination of hippuric acid is observed the possibility of this source must be taken into account.

Very little is known of the pathological variations in the excretion of hippuric acid; this is principally owing to the fact that until recently suitable methods for its quantitative estimation were not available. It is an interesting fact that, in accordance with Bunge's experiment in dogs, the formation of hippuric acid appears to be suspended in cases of acute as well as chronic parenchymatous nephritis, for the benzoic acid which is then ingested reappears in the urine unchanged. In amyloid degeneration a marked diminution has likewise been demonstrated. Large quantities of hippuric acid, on the other hand, have been noted in acute febrile diseases, hepatic diseases, diabetes mellitus, chorea, etc. The data, however, are insufficient to warrant any definite conclusions.

Quantitative Estimation of Hippuric Acid.—Folin's Method.—This is based upon the cleavage of the hippuric acid with caustic alkali and the subsequent extraction and estimation of the liberated benzoic acid, from which in turn the corresponding quantity of hippuric acid may be calculated.

One hundred c.c. of urine are treated in an evaporating dish with 10 c.c. of a 5 per cent. solution of sodium hydrate and evaporated to dryness on a steam-bath (overnight). The residue is transferred to a 500 c.c. Kjeldahl flask by the aid of 25 c.c. of water and 25 c.c. of concentrated nitric acid; 0.2 gram of copper nitrate is added as also a couple of quartz pebbles or pearls when the mixture is boiled very gently for four and one-half hours over a micro-burner. The object of this step is to prevent subsequent emulsion and to remove the coloring matters of the urine.

The necks of the flasks are fitted with Hopkins' condensers made from large test-tubes which should fit rather loosely. This arrangement is shown in the accompanying diagram (Fig. 157). After cooling, the surface of the condensers is rinsed with 50 c.c. of water and the contents of the flasks transferred to a 500 c.c. separating funnel by the aid of a small amount of water (25 c.c.). The solution, measuring now 100 c.c., is saturated with ammonium sulphate (about

55 gms.) and extracted with four successive portions of chloroform (50, 35, 25, and 25 c.c.) by shaking. The combined portions are then treated with 100 c.c. of a saturated solution of pure sodium chloride, containing 0.5 c.c. of concentrated hydrochloric acid per liter. The mixture is well shaken, the chloroform drawn off into a dry 500 c.c. Erlenmeyer flask and titrated with $\frac{n}{10}$ sodium alcoholate, using four or five drops of phenolphthalein as indicator. Folin recommends that the first distinct end-point be taken, even though it fade on standing a short time.

The sodium ethylate solution is made by dissolving 2.3 grams of cleaned metallic sodium in one liter of absolute alcohol, and is then standardized by titrating it against purified benzoic acid in washed chloroform.

1 c.c. of the $\frac{n}{10}$ sodium alcoholate will indicate 0.0122 gram of benzoic acid = 0.0179 gram hippuric acid.

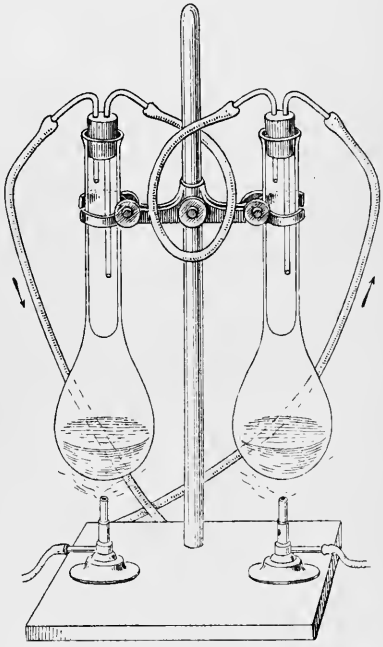


FIG. 157.—Arrangement showing Hopkins' condensers.

In adults creatin is supposedly only eliminated in the urine when much is ingested in the food or when there is an unusual disintegration of the body's own tissues (Folin). In children, on the other hand, the urine usually contains relatively large quantities, even under ordinary conditions (Rose, Folin), viz., 90 to 258 mg.

CREATIN AND CREATININ

The antecedents of creatin and creatinin are unknown. Two sources of the urinary creatinin must be recognized, viz., the muscle tissue of the body and the muscle tissue ingested as food. The tissue creatin is possibly transformed into creatinin and eliminated in this form, while the creatin which has been ingested does not appear in the urine as creatinin. Its fate is not known. Folin regards creatinin as the essential end product of the endogenous nitrogenous katabolism, in so far at least as the muscle tissue is concerned. He has demonstrated the interesting fact that its absolute quantity on a meat-free diet is a constant quantity, which is different for different

individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated. Its relative amount is increased when the urea nitrogen falls. On a diet rich in proteins the creatinin nitrogen represents 3.2 to 4.5 per cent. of the total, while on one free from proteins (starch and cream) the amount may rise to 17.4 per cent. The absolute amount seems to depend to a certain extent upon the body weight. Fat or corpulent persons yield less creatinin per unit of body weight, namely, 20 mg. per kilo, while lean persons yield about 25 mg.; 1.15 to 1.6 grams may thus be regarded as average values.

The study of pathological variations in the amount of creatinin has been greatly facilitated through the introduction of Folin's method (see below). The older data are of little importance unless the diet of the individual has been carefully considered. A diet rich in meats, it should be borne in mind, greatly increases the amount.

If, then, in patients affected with acute febrile diseases, such as pneumonia, typhoid fever, etc., a large increase is observed, the patient being at the same time upon a milk diet, an increased destruction of muscle tissue may be inferred, as a milk diet in itself, *cæteris paribus*, causes a diminished elimination. A decrease would logically be expected to occur during convalescence from such diseases. In the various forms of anemia, marasmus, chlorosis, phthisis, etc., a diminished amount is observed. The same is seen in advanced cases of chronic parenchymatous nephritis, in progressive muscular atrophy, in pseudohypertrophic paralysis, and in progressive ossifying myositis.

Quantitative Estimation.—Folin's Method.—This method is based on Jaffé's reaction of creatinin with alkaline picric acid solution. The red-colored solution produced in this reaction has in proper concentration and when viewed by transmitted light exactly the same shade as a potassium bichromate solution. Half-normal potassium bichromate solution (containing 24.55 grams per liter) is, therefore, used as a standard for comparsion. A high-grade colorimeter, by means of which the depths both of the unknown solution and of the bichromate can be adjusted to tenths of millimeters, is necessary for the comparison.[1]

The following solutions are also necessary: The half-normal potassium bichromate solution, 10 per cent. sodic hydrate, and a saturated (1.2 per cent.) picric acid solution.

If to 10 mg. of chemically pure creatinin dissolved in 10 c.c. of water in a 500 c.c. volumetric flask are added 15 c.c. of picric acid solution and 5 c.c. of sodic hydrate, the maximum color is obtained at the end of five minutes. If at the end of this time the solution be diluted to the 500 c.c. mark and at once compared with the

[1] The French instrument of Duboscq, which can be obtained through Eimer & Amend, is admirably suited for the purpose.

standard bichromate solution, it will be found that 8.1 mm. of the creatinin-picrate solution have in the colorimeter exactly the same shade and depth of color as 8 mm. of the bichromate solution.

The actual determination in urine is carried out in exactly the same way, substituting 10 c.c. of urine for the creatinin solution. The more creatinin that is present in the 10 c.c. of urine the deeper will, of course, be the color of the solution obtained. Supposing the colorimetric observation shows that 7.1 mm. of the urine-picrate solution are equal in color to 8 mm. of the standard, the 10 c.c. of urine would then contain $10 \times \frac{8.1}{7.1} = 11.4$ mg. of creatinin.

The following precautions are to be observed in the determination:

1. Make first a preliminary colorimetric observation, using half-normal potassium bichromate solution in both cylinders of the colorimeter, adjusting one to the 8 mm. mark. The average of three or four readings of the other cylinder should also be 8 mm., and after the first observation no two should differ by more than 0.2 mm. This preliminary observation takes only two or three minutes, and is exceedingly useful in making the eye sure of the correct point to be ascertained.

2. Exactly 8 mm. of the half-normal potassium bichromate solution must be used as the standard for comparison; 16 or 24 mm., for example, cannot be substituted on the basis of the calculation given above because the creatinin-picrate solution absorbs light at an entirely different rate from that of the bichromate solution.

3. For the reason given in the preceding paragraph it is necessary to make each determination with a quantity of urine containing not less than 5 nor more than 15 mg. of creatinin. Within these limits the determination as described is correct within 0.2 mg.

4. Sugar and albumin do not interfere with the determination. Acetone, diacetic acid, and hydrogen sulphide do interfere. Where these are present the urine should be measured into a porcelain evaporating dish and heated on a water bath with 10 c.c. of 1 per cent. hydrochloric acid for about half an hour. When the dish is again cooled, the reagents are added directly into the dish, and finally rinsed into the volumetric flask after five minutes.

5. The color due to the urine is ordinarily of no appreciable consequence because of the great dilution. Urines containing bile pigments can, however, first be cleared by the addition of egg albumin and then removing this by coagulation (heat).

The whole operation can be finished in less than fifteen minutes; indeed, it should be finished at once, as the colored product obtained by the interaction of creatinin and picric acid is not very stable.

OXALIC ACID

The origin of oxalic acid in normal urine is twofold. The greater portion is supposedly derived from the ingested food, but there is evidence to show that a certain amount is also formed during the metabolism of the body tissues, as the elimination of oxalic acid does not cease during starvation. The carbohydrates and fats probably do not play a part in this connection; and, according to Salkowski, the albumins also do not enter into consideration *per se*. He rather inclines to the view that the nucleins represent the antecedent of the oxalic acid, and, as a matter of fact, uric acid, which, as we have seen, is itself derived from the nucleinic bases, can be readily oxidized to oxalic acid, with the intermediary formation of parabamic acid and *oxaluric acid*. The latter has been repeatedly demonstrated in the urine, and it is conceivable that the same process may occur in the animal body. But even supposing that the oxaluric acid which is obtained from the urine is formed artificially during the lengthy process of analysis, and that the substance did not exist preformed, there is no reason for the assumption that uric acid may not be the normal antecedent of the oxalic acid. For Salkowski has demonstrated conclusively that on oxidation with ferric chloride in aqueous solution uric acid yields oxalic acid and urea directly.

The matter, however, is not quite so simple as it appears, and an increased elimination of oxalic acid by no means always occurs when the output of uric acid is increased. After the ingestion of fairly large amounts of thymus, for example, the usual increase of uric acid is not accompanied by a corresponding increase in the amount of oxalic acid, and in those cases in which it does occur we are as yet unable to exclude the large amount of connective tissue as the source of the oxalic acid. Connective tissue and gelatin have, as a matter of fact, been shown to increase the amount of oxalic acid when given in large amounts. With pure nuclein no effect has been observed, and it can be shown that in those experiments in which this was used by mouth an absorption from the intestinal tract had manifestly not occurred (Mohr and Salomon).

Under pathological conditions oxalic acid may also be formed in the digestive tract from the ingested carbohydrates, as a result of a peculiar fermentative process. This has been well shown by Helen Baldwin in Herter's laboratory. In some of these cases no free hydrochloric acid could be demonstrated in the gastric contents, and it was observed that inoculation of a digestive mixture, which was originally free from oxalic acid, resulted in its appearance if a few drops of such stomach contents were added. In dogs prolonged feeding with excessive quantities of glucose, together with meat, was seen to lead eventually to a state of oxaluria, which was

associated with a mucous gastritis and the absence of free hydrochloric acid. Oxalic acid could then also be demonstrated in the stomach contents. Very curiously the ingestion of quite small and non-toxic amounts of oxalic acid is followed by a fairly intense indicanuria.

The amount of oxalic acid which is normally eliminated in the twenty-four hours fluctuates with the amount ingested, and varies from a few milligrams to 2 or 3 centigrams, being usually less than 10 milligrams (Baldwin). It is influenced by the character of the diet. The ingestion of oxalates by the mouth is followed by their partial elimination only in urine and feces, so that we may conclude that to a certain extent oxalic acid is decomposed during its passage through the animal body; possibly this may occur in the intestinal canal as the result of bacterial action.

Foods rich in oxalic acid are spinach, tomatoes, carrots, celery, string-beans, rhubarb, potatoes, dried figs, plums, strawberries, cocoa, tea, coffee, and pepper. Foods which contain little or no oxalic acid, on the other hand, are meat, milk, eggs, butter, cornmeal, rice, peas, asparagus, cucumbers, mushrooms, onions, lettuce, cauliflower, pears, peaches, grapes, melons, and wheat, rye, and oat flour.

Before drawing conclusions as to the existence of abnormal oxaluria, it is hence imperative to eliminate the possibility of an increased ingestion, by placing the patient upon a diet which contains little or no oxalic acid.

An increased elimination is notably observed in association with various dyspeptic and nervous manifestations, and constitutes the condition commonly spoken of as the *oxalic acid diathesis*, or as *idiopathic oxaluria*. Its existence as a definite pathological picture is, however, denied by most modern clinicians. Nevertheless, it must be admitted that there is a certain type of neurasthenia in which, generally in association with hyperchlorhydria, an increased elimination of oxalic takes place, and in which a copious deposit of calcium oxalate crystals is frequently observed. From the mere fact of the occurrence of such deposits, of course, no inference is, as a rule, to be drawn regarding the actual elimination, but its frequent occurrence is in itself of importance, as in such cases a similar separation from the urine may already occur within the urinary passages, and not uncommonly in the pelvis of the kidneys. Not infrequently oxaluria of this type is associated with an increased elimination of uric acid and a mild grade of albuminuria, as has been shown by Senator, von Noorden, Da Costa, myself and others. Whether or not the oxaluria in these cases can be explained upon the basis of abnormal fermentations in the gastro-intestinal tract, as is suggested by the observations of Baldwin remains to be seen. In some this may be the case, but in others I am inclined to associate the oxaluria with the coexistent lithuria.

Very interesting is the apparently vicarious oxaluria which is at

times observed in diabetes. Fürbringer has reported a case of diabetes in which the elimination of oxalic acid was described as "enormous," and in which oxalic acid could also be demonstrated in the sputum (oxaloptysis). Rausch has recorded a case of mild diabetes, associated with hepatic cirrhosis, in which 1.2 grams were excreted in twenty-four hours. In most cases of diabetes, on the other hand, an increased oxaluria cannot be demonstrated.

In cases of obesity Kisch found no abnormal degree of oxaluria.

In association with jaundice increased oxaluria has been repeatedly observed, and is probably referable to biliary stasis and consequent cholemia, as Salkowski has demonstrated that the bile contains oxalic acid. In pneumonia and leukemia, in both of which we find, as a rule, a greatly increased elimination of uric acid, the oxalic acid is not always increased, and sometimes, indeed, quite low in comparison with the amount of uric acid.

Quantitative Estimation.—Heretofore the old method of Neubauer has been in general use, but it is at best unsatisfactory. It has been replaced by the method of Dunlop.

Dunlop's Method (Slightly Modified by Baldwin).—In this case the calcium oxalate is precipitated from an acid solution by means of alcohol, instead of from an alkaline solution by calcium chloride. The urine is thymolized, and, if alkaline, acidified with a trace of acetic acid; 500 c.c. of a well-mixed specimen of the collected urine of twenty-four hours are treated with 150 c.c. of over 90 per cent. alcohol, to precipitate the calcium oxalate. The mixture is set aside for forty-eight hours. It is then filtered, care being taken to insure the entire removal of the crystals from the beaker. The sediment is thoroughly washed with hot and cold water, and finally with dilute acetic acid (1 per cent. solution). The filter is placed in a small beaker and soaked in a small amount of dilute hydrochloric acid. It is then washed with hot water until the washings no longer give an acid reaction. The acid solution and washings are filtered, and the filtrate evaporated to about 20 c.c. This is treated with a very small amount of a solution of calcium chloride, to insure the presence of an excess of calcium. The solution is neutralized with ammonia, slightly acidified with acetic acid, and treated with strong alcohol, so that the mixture contains 50 per cent. After forty-eight hours the sediment is collected on a filter free from mineral ash, and is washed with cold water and dilute acetic acid until free from chlorides. The filter with its contents is then incinerated, first over a Bunsen burner, and afterward for five minutes in a blow-pipe flame. On cooling over sulphuric acid the ash is weighed; the result multiplied by 1.6 represents the amount of oxalic acid in the volume of urine examined.

ALBUMINS

The albumins which may be met with in the urine are serum albumin, serum globulin, albumoses (peptones), the albumin of Bence Jones, hemoglobin, nucleo-albumin, fibrin, histon, and nucleo-histon. Of these, serum albumin is the most important from a clinical standpoint.

Serum Albumin.—The question whether or not serum albumin occurs normally in the urine—*i. e.*, under strictly physiological conditions—has been much disputed. It is claimed by some that traces may be temporarily met with in apparently healthy individuals after severe muscular exercise, cold baths, mental labor, severe emotions, during menstruation, digestion, etc. This so-called *physiological albuminuria* mostly occurs in young adults, and is usually, if not always, of brief duration. The urine, it is claimed, is otherwise normal—*i. e.*, of normal amount, appearance, specific gravity, and composition, and free from abnormal morphological constituents, such as casts, red corpuscles, leukocytes, and epithelial cells. However, Darling has shown that severe muscular exercise may produce a urinary picture which, even though temporary, closely simulates what is seen in acute nephritis. He reports 0.9 per cent. of albumin in a member of a Harvard four-oared crew after a two-mile race, and amounts varying from 0.25 to 0.5 per cent. in five others under similar conditions. The sediment at the same time contained large numbers of hyaline and finely granular casts, many with renal cells and red blood corpuscles adherent. In many of the sediments there were also numerous red cells as such and an excess of leukocytes.

The existence of a physiological albuminuria, on the other hand, is denied, and the occurrence of serum albumin at least regarded as pathological in every case. I have never been able to convince myself of the occurrence of serum albumin in the urine under strictly physiological conditions, and am hardly prepared to regard severe muscular and mental labor, severe mental emotions, cold baths, etc., as physiological stimuli. The albuminuria, so often observed during the first days of life, at which time sediments of uric acid and urates, mucus, epithelial cells from the different portions of the urinary tract, and even casts may also be seen—*i. e.*, constituents which in adults would rightly be regarded as abnormal—has also been brought forward in support of the theory of a physiological albuminuria. There can be no doubt, however, that this form of albuminuria is referable to the profound changes that take place in the circulatory system after birth, and to some extent perhaps also to the well-known uric acid infarctions so frequently seen in the kidneys of the newly born, so that it would probably be better and more in accord with the teachings of pathology to regard this form of albuminuria also as abnormal.

The more closely the subject of the so-called physiological albuminuria is studied the more improbable does its physiological nature appear, and a more detailed study of these cases, it may be confidently asserted, will ultimately lead to the conclusion that *the presence of albumin in every case is a pathological phenomenon.* In this connection it is interesting to note, however, that the experience of life insurance companies has been that but few of those who have shown simple albuminuria in the past have any evidence of nephritis ten years later.

The association of an increased elimination of urea and a constant tendency toward the deposition of uric acid sediments with albuminuria in apparently healthy individuals was noted many years ago, but received comparatively little attention. Personal observations have led me to look upon this form of albuminuria as of common occurrence, and while in almost every case the albumin can be caused to disappear from the urine by proper diet and exercise, there can be no doubt that, if neglected, degenerative changes in the kidneys may ultimately result.

An albuminuria may at times be observed in anemic children and adolescents, and particularly in masturbating boys of the mouth-breathing type, but can hardly be regarded as physiological. The same may be said of the albuminuria of pregnancy and parturition.

As regards the action of cold baths, Rem-Picci reports that albuminuria may be considered a constant phenomenon after cold baths, but that different subjects react differently under the same conditions. Those which show albuminuria more readily are, as a rule, the less robust and thinner individuals, who are especially sensitive to cold. The limits of temperature necessary to produce the phenomenon are from 12° to 13° C., when the immersion is not longer than three minutes. If the temperature be from 15° to 20° C., the albumin appears only after fifteen minutes' immersion. Above this temperature albuminuria does not occur, even if the bath lasts much longer. The colder the bath the more rapid the appearance of albumin. The degree of albuminuria is always slight, and even in the more marked cases rarely exceeds 0.25 pro mille. The sediment, according to Rem-Picci, occasionally shows a few hyaline casts, and often crystals of calcium oxalate.

The course which may be taken by these various forms of what should be termed *functional* albuminuria, in which the amount of albumin rarely exceeds 0.1 per cent., is very interesting. The elimination of albumin may thus be quite *transitory* on the one hand, as when following severe muscular exercise, cold baths, and the like. It may, however, also last for several days, or even weeks, and be followed by a disappearance of the albumin for a variable length of time, and again by its reappearance and continuance for days and weeks. The term *intermittent albuminuria* has been applied to this

latter type. At times the albuminuria may follow a definite course, disappearing and reappearing with such regularity that it has not improperly been styled *cyclic albuminuria.* In this form the albumin generally disappears from the urine during the night or during prolonged rest in bed, and reappears during the day, the erect posture apparently favoring its reappearance; the term *postural* or *orthostatic albuminuria* has hence also been suggested for this form. Oswald, who made a careful study of cyclic albuminuria in Riegel's clinic, regards its occurrence as distinctly pathological, and as indicating the existence of nephritis. Remembering the importance of the subject, it may not be out of place to enumerate the reasons which led Oswald to this conclusion:

1. The patients generally come to the physician complaining of certain definite symptoms which are similar to those noted in cases of true nephritis. At times, however, no complaints are made, because the patients have reasons for concealing them (as in examinations for life insurance), or because they are temporarily absent.

2. The subjective complaints, as well as the anemia so frequently observed in such cases, generally disappear, together with the albumin, under suitable treatment, and reappear when the anemia again becomes marked.

3. In many a history of an antecedent nephritis the result of scarlatina or diphtheria may be obtained, as in 3 cases of Heubner, in 14 cases out of 20 described by Johnson, etc. In some also a direct transition from an acute nephritis to the cyclic form of albuminuria has been noted. Where this was not possible the history of an acute infectious disease or an angina that had been overlooked in the clinical history must be regarded as a possible cause.

4. The absence of morphological elements, especially tube casts, does not exclude a nephritis. A large number of cases, moreover, have recently been observed in which casts were repeatedly found.

5. A cyclic albuminuria may be observed in many cases of chronic nephritis.

6. Marked organic abnormalities (such as heart lesions) need not be demonstrable, as they may be absent for a long period of time or may be unrecognizable.

A certain type of orthostatic albuminuria has of late been brought into correlation with lordosis (*lordotic albuminuria*), and it is interesting to note that in some of these cases the albuminuria can be induced by having the patients assume the lordotic posture in a chair for fifteen minutes. Coincidentally with the appearance of the albumin or its increase, an increase in the acidity of the urine has been noted and, according to Fränkel, it is possible to suppress the albuminuria resulting from lordotic posture by a previous administration of bicarbonate of sodium.

According to the researches of Erlanger and Hooker orthostatic

albuminuria is dependent upon a lowering of the pulse pressure (being the difference between the minimum and the maximum blood pressure), which constantly occurs when the individual changes from the recumbent to the erect posture.

In the true form of orthostatic albuminuria the albumin present is serum albumin Casts are absent.

It may be safely asserted that a transitory, intermittent, and cyclic albuminuria is not infrequently observed in apparently healthy individuals, but that the facts so far brought forward do not warrant the assumption that such forms of albuminuria are physiological. The occurrence of such albuminuria unquestionably demonstrates a certain insufficiency of the renal epithelium, and I am much in favor, as Martius has proposed, of discarding the term *physiological* albuminuria altogether, and to speak of these various forms collectively as *constitutional* albuminuria.

The different forms of albuminuria which may be observed under pathological conditions may be grouped under the following headings:

1. **Albuminuria Associated with Organic Diseases of the Kidneys,** viz., acute and chronic nephritis, renal arteriosclerosis, amyloid degeneration of the kidneys.

In acute nephritis, albuminuria, usually of great intensity, is a constant and most important symptom. The amount eliminated is generally proportionate to the intensity of the disease, but varies within fairly wide limits, generally from 0.3 to 1 per cent., corresponding to a daily excretion of from 5 to 8 grams. Much larger quantities, it is true, are at times excreted, but it may be definitely stated that the daily loss of albumin seldom exceeds 20 grams.

In chronic parenchymatous nephritis the elimination of albumin is likewise constant, and the amount excreted in severe cases may even exceed that observed in the acute form. An elimination of from 15 to 30 grams, viz., 1.5 to 3 per cent. by weight, is frequently observed.

In the ordinary form of chronic interstitial nephritis the elimination of albumin is, as a general rule, slight, and rarely amounts to more than 2 to 5 grams pro die. At the same time it is not unusual to meet with an apparent absence of albumin if the more common tests (see below) are employed. If it is remembered that very often the diagnosis of the disease is dependent upon the demonstration of the presence or absence of albumin, the necessity of *frequent* examinations and the employment of more delicate tests, particularly of the trichloracetic acid test, as well as of a microscopic examination, is at once apparent. This is even of greater importance in the renal arteriosclerosis of Senator, in which albumin by the ordinary tests is probably not demonstrable in the majority of cases, and in which even the trichloracetic acid test *may* not be of service, and casts be absent.

Amyloid degeneration of the kidneys, in the absence of inflammatory processes, is accompanied by a condition of the urine closely resembling that observed in the ordinary form of chronic interstitial nephritis. A total absence of albumin, however, is less frequently noted, while an amount varying between 1 and 2 per cent. is not uncommon. It will be shown later on that in this condition considerable amounts of serum globulin are excreted in addition to the serum albumin; larger amounts, in fact, than are generally observed in this form of chronic renal disease; so that Senator suggests that such a relation, in the absence of an acute nephritis, or an acute exacerbation of a chronic nephritis, may be of a certain diagnostic value.

2. **Febrile Albuminuria.**—That albuminuria may occur in almost any one of the various febrile diseases is a well-known fact, but it is important to remember that, while such an albuminuria *may* at times be referable to a true nephritis developing in the course of or during convalescence from an acute febrile disease, such is the exception, and not the rule. Under this heading we are considering that form only which is not associated with distinct changes affecting the renal parenchyma, and which generally appears during the height of the disease only, and disappears with a return of the temperature to normal. As has been mentioned, it is often difficult, if not impossible, to assign a definite cause for an albuminuria of this character, and in all probability, several factors are in operation at the same time. In the beginning of the disease, when the blood pressure, as a rule, is increased, the albuminuria may be referable to an ischemia of the kidneys, as the increased pressure in fever, according to Cohnheim and Mendelson, is largely referable to spasm of the arterioles. Later on, or in the beginning of cases in which especially severe intoxication exists, the blood pressure may be subnormal, and the albuminuria be due to this cause—*i. e.*, a hyperemic condition of the kidneys. As a matter of fact, it has been experimentally demonstrated that both anemia and hyperemia of the kidney structure may lead to albuminuria. On the other hand, it is not unlikely that the strain thrown upon the kidneys by an excessive elimination of organic material, in the absence of a correspondingly large quantity of water, may produce albuminuria. I have repeatedly seen the functional albuminuria of the type described by Da Costa disappear during the administration of a diet relatively poor in nitrogen, while an increased diuresis was at the same time effected by the consumption of large amounts of water.

In those grave cases of typhoid fever, furthermore, which are characterized by high fever and pronounced nervous symptoms, it would appear quite likely that the albuminuria, which in these cases is particularly marked, is referable to a direct influence upon the central nervous system, and in some cases, at least, also dependent

upon an irritant action upon the renal epithelium on the part of the microbic poisons circulating in the blood. The character of the albuminuria will largely depend upon the intensity of the intoxication; in other words, upon the amount of bacterial poison present at any one time in the blood.

3. **Albuminuria Referable to Circulatory Disturbances.**—To this class belongs the albuminuria so frequently observed in cardiac insufficiency referable to valvular lesions, degeneration of the heart muscle from whatever cause, disease of the coronary arteries, etc., as well as in cases of impeded pulmonary circulation affecting the general circulation through the right heart, and, finally, in conditions associated with local circulatory disturbances, such as compression of the renal veins by a pregnant uterus, tumors, etc. It has been pointed out that febrile albuminuria also may, to a certain extent at least, be referable to such causes—*i. e.*, an ischemia or hyperemia of the kidneys produced by an increased or diminished blood pressure. The albuminuria observed in cases of cholera infantum, the simpler forms of intestinal catarrh, and in cholera Asiatica particularly, are undoubtedly dependent upon such causes. The quantity of albumin found under these circumstances varies considerably, but rarely exceeds 0.1 to 0.2 per cent. unless the disease has advanced to a stage where distinct changes in the renal parenchyma have resulted. The occurrence of albuminuria after cold baths, as stated above, is regarded by many as a "physiological" phenomenon, but this view should be rejected, as there can be little doubt that this form is also referable to circulatory disturbances.

4. **Albuminuria Referable to an Impeded Outflow of Urine.**—Clinically, albuminuria referable primarily to an impeded outflow of urine from the kidneys is probably of more frequent occurrence than is generally supposed, and especially in women, in whom Kelly and others have demonstrated the frequent existence of ureteral stenoses. A complete blocking of the excretory duct, on the other hand, is rarely seen, but may be caused by the impaction of a renal calculus, the pressure of a tumor, or following certain gynecological operations in which the ureter is accidentally caught in a suture, etc. It has also been suggested that the albuminuria of pregnancy may be due to a compression of a ureter, but it is more likely that other factors are here at play.

5. **Albuminuria of Hemic Origin.**—Clinically, albuminuria of hemic origin is observed in various diseases of the blood, such as purpura, scurvy, leukemia, pernicious anemia, as also in cases of poisoning with lead and mercury, in syphilis, jaundice, diabetes, following the inhalation of ether and chloroform, etc.

6. **Toxic Albuminuria.**—It has already been stated that the albuminuria of acute febrile diseases may, to a certain extent, be referable to a direct irritant action of bacterial poisons upon the renal

parenchyma. Poisoning with cantharides, mustard, oil of turpentine, potassium nitrate, carbolic acid, salicylic acid, tar, iodine, petroleum, phosphorus, arsenic, lead, antimony, alcohol, and mineral acids produces albuminuria.

7. **Neurotic Albuminuria.**—It is claimed by some that albumin, usually in small amounts, is eliminated in epilepsy after every attack, while others either deny its occurrence under such conditions or regard it as exceptional. In a number of cases in which I had occasion to examine urine voided after an attack, albumin was usually absent. It should be stated, however, that the seizures in these cases were comparatively slight, and that unfortunately an examination for semen was not made in those urines in which traces of albumin were demonstrated. An examination of the urine voided by a patient, after having been in the epileptic state for more than forty-eight hours, showed the presence of a small amount of albumin. Semen was absent. Nothnagel states that he could not demonstrate any regularity in the appearance of albumin. In some of his cases with major attacks there was no albumin; in others it appeared after every attack; in still others it was sometimes present and at other times absent (in the same individual). At times it was found after a minor attack and was absent after a major attack (also in the same individual).

Other observers have obtained similar results, so that we may conclude that albuminuria following epileptic seizures is rather the exception than the rule. When it does occur, its significance is essentially the expression of a certain grade of cyanosis during the attacks.

A transient albuminuria has also been noted in cases of progressive paralysis, mania, tetanus, delirium tremens, apoplexy, migraine, Basedow's disease, brain tumor, etc.

Although albuminuria may apparently be produced artificially by injuries affecting a certain area in the floor of the fourth ventricle analogous to the production of glucosuria (see Glucosuria), it would probably be going too far to assume the existence of a certain specific centre, stimulation of which causes the appearance of albumin in the urine. While the influence of the nervous system in preventing the passage of albumin through the glomeruli under normal conditions is undoubted, it would appear more likely that the albuminuria following injuries to the central nervous system is referable to circulatory disturbances in the kidneys secondary to lesions of the brain, and especially of the medulla. The albuminuria observed in certain neurotic individuals, on the other hand, is probably more frequently associated with metabolic abnormalities, and of "constitutional" origin.

8. **A Digestive Albuminuria** also has been described. It may follow the ingestion of excessive amounts of cheese, eggs—particu-

larly when taken raw—beef, etc. Specially interesting is the form which follows the ingestion of excessive amounts of egg albumin. Ordinarily the consumption of a moderate amount of such albumin does not lead to albuminuria, while in cases of nephritis an already existing albuminuria is increased. But it has also been noted that even in individuals with *apparently* healthy kidneys the ingestion of an excessive amount of egg albumin may call forth albuminuria, and it is possible in both cases to demonstrate the presence in the urine of both egg albumin and blood albumin.

To examine into this question the individual is given from four to eight raw eggs on an empty stomach in the morning for two to four days. His diet otherwise is as usual. The urine is collected at intervals of from two to three hours. If the ingestion of such an amount of egg albumin leads to albuminuria, this usually occurs after about four hours, and reaches its maximum intensity two hours later. Casts are not found (Jnouye).

The albuminuria in question, so far as the egg albuminuria goes, is undoubtedly owing to the fact that a certain amount of egg albumin is absorbed as such from the gastro-intestinal canal and is subsequently eliminated as foreign material. In what manner, however, the egg albuminuria may be responsible for the accompanying serum albuminuria is more difficult to explain.

Of the albuminuria which follows excessive indulgence in cheese and beef but little is known. Bearing in mind that the albuminuria very often follows the ingestion of such articles almost immediately, and before they have become absorbed, it is hardly justifiable to refer this form to the existence of a hyperalbuminosis.

In the account thus given of the occurrence of albuminuria and its possible causes, reference has been had to only a *purely renal* albuminuria. It should be remembered, however, that the origin of the albumin may often be extremely difficult to determine, as albuminous material, such as blood and pus, may become mixed beyond the glandular portion of the kidneys with what would otherwise have been a perfectly normal urine, and that such an admixture may take place not only in the ureters, the bladder, and the urethra, but even in the pelvis of the kidney.

The term *accidental albuminuria* is applied to a condition in which albuminous material becomes mixed with a urine beyond the kidneys, as in cases of cystitis and urethritis, or whenever semen has entered the urine while the renal urine proper is free from albumin. An admixture of pus, blood, lymph, or chyle may, however, also occur in the kidneys, when the albuminuria is termed *accidental renal albuminuria*, an example of which is frequently seen in the slight degree of albuminuria referable to pyelitis during convalescence from typhoid fever. By a *mixed albuminuria* and a *mixed renal albuminuria*, on the other hand, we are to understand conditions in which the source

of the albumin is twofold, renal and extrarenal in the first instance, parenchymal and extraparenchymal in the second, examples being the albuminuria of cystitis combined with nephritis and pyelonephritis respectively.

It is manifest, of course, that in every instance in which albumin is found in the urine its origin should be ascertained. While this question is usually readily decided by a microscopic examination of the urine, considerable difficulty may occasionally be experienced. It is a well-known fact that in the urine of women a trace of albumin may frequently be detected, which is not due to any lesion of the urinary organs, but to an admixture of vaginal discharge or of blood during the process of menstruation. Whenever, therefore, doubt is felt as to the origin of the albumin, the specimen for examination should be obtained by the catheter. In men albumin may be referable to a gonorrheal urethritis. In such cases it is well to let the patient flush out his urethra first, and to make use for examination of the portion last voided. Very often, however, the conditions are more complex, it being uncertain whether the albumin is referable to the presence of pus only, or whether its origin is in the renal parenchyma. In such cases, as in cystitis, pyelonephritis, etc., a careful microscopic examination and enumeration of the pus corpuscles with the Thoma-Zeiss instrument are called for, and will in the majority of instances decide the question. Generally speaking, the amount of albumin found in uncomplicated cases of cystitis does not exceed 0.15 per cent., while in cases of pyelitis of the same intensity the amount of albumin is from two to three times as large.

Of late, attention has repeatedly been drawn to the occasional presence in the urine of the albuminous body which is soluble in acetic acid, and which Patein regards as a modification of common serum albumin. It has thus far been observed in only 8 cases, viz., twice in chronic nephritis, three times in eclampsia, once in a cystic kidney, once in tonsillitis following an injection of diphtheria antitoxin, and once in a pregnant woman in whom typhoid fever developed. I should sugest that the substance be spoken of as *Patein's albumin* until its chemical identity has been established. The term *acetosoluble albumin* is, of course, likewise admissible.

So far as the *amount of albumin* is concerned which may be eliminated in the twenty-four hours, an excretion of less than 2 grams may be regarded as insignificant, 6 to 8 grams as a moderate amount, and 10 to 12 grams or more as excessive. An excretion of 20 to 30 grams is exceptional.

Serum Globulin.—It has been pointed out that in cases of amyloid degeneration of the kidneys serum globulin is found in the urine together with serum albumin in large amounts, and, according to Senator, a ratio between the two albumins of 1 to 0.8 to 1.4 may be

regarded as a fairly constant symptom of the disease, and of diagnostic importance. There seems to be no doubt, however, that serum globulin occurs in the urine, although in much smaller quantities than in the disease mentioned, whenever serum albumin is eliminated.

Hoffman designates as the *albumin quotient* the amount of serum albumin divided by the amount of serum globulin. In most cases of albuminuria this varies between 1.5 and 2.3, the amount of globulin being the variable factor. According to Oswald euglobulinuria is the mildest form of albuminuria. In contracted kidney and chronic passive congestion the quotient lies between 2.8 and 5.3, while in amyloid disease it may be lower than 1. The lowest values probably are seen in acute nephritis.

A most remarkable instance of globulinuria has been recorded by Noel Paton, in which the globulin separated out in crystalline form and was found in extraordinarily large quantity, amounting in one day to 70 grams. (See Bence Jones albumin.)

Albumoses.—Albumoses have frequently been encountered in the urine, but are probably more frequently overlooked, as the bodies in question are not precipitated on boiling.

Albumosuria is observed under a great variety of conditions. It is thus noted in association with large accumulations of pus within the body, and there can be little doubt that the albumosuria is in such instances referable to a disintegration of the pus corpuscles and a resorption of the resulting albumoses. This form has hence been termed *pyogenic albumosuria*. It is principally observed during the stage of resolution in cases of croupous pneumonia; in association with pyothorax, and in cases of epidemic cerebrospinal meningitis, as contrasted with the tubercular form. A *hepatogenic form* is noted in connection with diseases of the liver, notably acute yellow atrophy. Of its origin, however, nothing is known. Formerly, when the condition was looked upon as a peptonuria, and when it was thought that peptones were retransformed into native albumins in the liver, the "peptonuria" was explained upon the assumption that the liver had lost this power, and that the "peptones" accumulated in the blood, and were consequently eliminated in the urine. At the present day this view is no longer tenable.

An *enterogenic form* of albumosuria has been noted in various diseases of the intestinal tract, such as typhoid fever, tubercular ulceration, carcinoma, etc.; and it is possible that in these cases the albumoses are either directly absorbed from disintegrating pus, or that the intestine perhaps has in part lost the power of preventing the resorption of albumoses as such into the blood.

A *histogenic* or *hematogenic* origin has been ascribed to the albumosuria which is seen in cases of scurvy, in dermatitis, in various forms of poisoning, during the puerperal period and pregnancy, par-

ticularly following the death of the fetus, in various psychoses, in cases of carcinomatosis, acute yellow atrophy, etc.

A *renal* or *vesical form* of albumosuria is further noted in which the albumoses are derived from contained albumins, owing either to the presence of the common proteolytic ferments of the urine or to bacterial action, as in decomposing albuminous urines.

Aside from the conditions already mentioned, albumosuria has been observed in various septic conditions, in diphtheria, measles, scarlatina, acute articular rheumatism, mumps, malaria, phthisis; further, in association with leukemia, nephritis, puerperal parametritis, endocarditis, caries, pleurisy, heart disease, apoplexy, myxedema, carcinomatous peritonitis, in pneumonia, at the height of the disease and before resolution has set in, in liver abscess, etc.

In the differential diagnosis of suppurative meningitis a positive albumose reaction, according to Senator, speaks strongly in favor of the existence of this disease. In support of this view he cites the case of a young man, the subject of a median otitis of long standing, in which symptoms pointing to a meningitis—viz., fever, headache, and pains in the neck—were present, but in which no albumosuria was found to exist, and in which an operation revealed the presence of a cholesteatoma. A *digestive form* of albumosuria has recently been described, in which albumoses appear in the urine after their ingestion in large quantities, and it is claimed that this is observed only in cases of ulcerative disease of the intestinal tract. Only a positive result, however, is of value.

Very frequently albumosuria accompanies albuminuria, a condition which Senator has termed *mixed albuminuria,* and it is interesting to note that the albumosuria may alternate with the albuminuria and may precede or follow the latter. In any case in which albumoses can be demonstrated in the urine the appearance of albumin should accordingly be anticipated.

In all cases of albumosuria the amount of albumose that appears in the urine is relatively small, and, as a rule, cannot be demonstrated by the biuret test when applied directly to the native urine. On the contrary, it is necessary to isolate the substance more or less definitely before deductions can be drawn as to its presence or absence.

Bence Jones' Albumin.—While the so-called Bence Jones' protein is notably met with in cases of multiple myeloma, and especially when affecting the thoracic skeleton, the substance is not strictly pathognomonic of this condition, as it has also been encountered in case of osteomalacia, lymphatic leukemia, carcinomatosis involving the osseous system, and in one case of gunshot wound of the leg. The condition is nevertheless more common in myelomatosis than in other diseases involving the bone marrow. On the other hand, cases of myelomatosis have been reported in which the protein in question could not be demonstrated, and it may disappear at a time

when the lesions in the marrow are most extensive. According to Boggs and Guthrie, moreover, the substance may appear before there is any demonstrable bone lesion (!).

Regarding the chemical nature of the substance but little is known. Bence Jones, its discoverer, as well as the earlier observers regarded it as an albumose. From the researches of Magnus Levy and my own investigations, however, it appears that the substance is in reality a true protein, as it yields a proto-albumose on peptic digestion; but it differs from all known proteins in its relative solubility on boiling, and in the readiness with which it dissolves in dilute ammonia after precipitation with alcohol. Like casein, it contains no hetero-group, but is distinguished from it by the presence of a carbohydrate radicle and the probable absence of phosphorus. It is crystallizable, and may occur in the urinary sediment in the form of typical spheroliths.

The amount of the substance which may be found in the urine is variable. Some observers have noted an elimination of from 0.25 to 6 pro mille, while others report much larger quantities. In Bence Jones' case the elimination rose on one occasion to 6.7 per cent., corresponding to a total output of 70 grams in the twenty-four hours—*i. e.*, to nearly as much as the entire amount of the proteins of the blood plasma.

As regards the origin of the protein nothing definite is known, but there is reason to suppose that it is not derived from the myelomatous tissue as such. We may imagine, however, that through the agency of the cells of the diseased tissue the formation of the normal blood proteins is impeded, resulting in the production of the substance in question, which is then eliminated as foreign matter.

As the diagnosis of myeloma, in its early stages at least, is altogether dependent upon the demonstration of the albumin in question, a special examination should be made in this direction in all cases of obscure bone pain, as also in obscure cases of anemia, since Ellinger has shown that at times the disease may take its course without the occurrence of local symptoms, while a marked anemia may exist. Of special interest in this connection is the fact that Zülzer claims to have succeeded in bringing about the appearance of Bence Jones' albumin in the urine of animals by feeding with pyrodin, which is known to be a hemolytic poison.

Peptonuria.—To judge from the investigations of Ito, peptone in the sense of Kühne, may occur in the urine under pathological conditions. He obtained positive results in pneumonia, in advanced cases of phthisis, in ulcer of the stomach, and in several women after childbirth. The reaction was most intense in the pneumonia cases; it appeared already before resolution occurred, and disappeared a few days after the crisis. In the parturient women no reaction was obtained if the examination was delayed until after

the tenth day. It is noteworthy that in the cases examined by Ito the peptonuria was always associated with the presence of albumoses (deutero-albumoses), and that the peptone was present in still smaller amount than the albumoses.

Hemoglobin (Methemoglobin).—Under normal conditions the disintegration of the red blood corpuscles which is constantly taking place in the body never results in such a degree of hemoglobinemia as to be followed by an elimination of hemoglobin in the urine. Whenever the destruction of red corpuscles is so extensive, however, that the liver is unable to transform into bilirubin all the blood-coloring matter set free, *hemoglobinuria* occurs. While these factors, then—*i. e.*, an excessive destruction of the red blood corpuscles and an insufficiency on the part of the liver—must be regarded as explaining every case of hemoglobinuria, our knowledge of the ultimate causes of such excessive disintegration, as well as the manner in which these operate, is limited. Formerly the term *hematinuria was* applied to this condition. It was shown, however, that the pigment eliminated is in reality not hematin, but usually methemoglobin, and only at times hemoglobin, so that the term hemoglobinuria also is ill chosen. Most common is the hemoglobinuria produced by certain poisons, such as potassium chlorate, arsenious hydride, hydrogen sulphide, pyrogallic acid, naphthol, hydrochloric acid, tincture of iodine, carbolic acid, carbon monoxide, etc., and also by morels (Helvella esculenta).

Hemoglobinuria is constantly observed following the transfusion not only of the blood of an animal of an alien species, but even of the blood of man, when either the donor or the recipient's serum is hemolytic for the corresponding corpuscles.

Quite common is the hemoglobinuria observed in connection with extensive burns and isolation.

While hemoglobinuria may occur in the course of any one of the specific infectious diseases, such as scarlatina, icterus gravis, variola hæmorrhagica, typhoid fever, yellow fever, etc., it is said to be especially frequent in cases of malarial intoxication. This view is not accepted by many; Osler, among others, believes that it has frequently been confounded with malarial hematuria. I have never seen an instance of malarial hemoglobinuria, and believe that in our more temperate zones it scarcely ever occurs. Bastianello asserts that it is likewise rare in Italy, but more common in Sicily and Greece, and very common in the tropics. According to the same observer, hemoglobinuria occurs only in infections with the estivo-autumnal parasite. A hemoglobinuria due to quinine is likewise said to exist, but is certainly rare; I have seen but one instance of the kind. To judge from the literature upon the subject, there can be no doubt that syphilis may under certain conditions be a factor in the production of hemoglobinuria. This appears to be particularly true of those cases of so-called paroxysmal hemoglobinuria in which

bloody urine is voided from time to time, the attacks being frequently preceded by chills and fever, so as closely to simulate malarial fever. Other factors, also, notably cold, appear to be concerned in the production of this form.

The occasional occurrence of hemoglobinuria in cases of Raynaud's disease, coincident with attacks of an epileptiform character, has been referred to in the chapter on the Blood.

Hemoglobinuria has been observed in a case of leukemia complicated by icterus.

Finally, an epidemic hemoglobinuria has been described as occurring in the newborn associated with jaundice, cyanosis, and nervous symptoms; of its causation we are in ignorance.

While hemoglobinuria is rather uncommon, *hematuria* is frequently observed, and will be considered later on, as its recognition is not dependent upon the demonstration of the albuminous body, "hemoglobin," alone in the urine, but upon the presence of red corpuscles, which in hemoglobinuria are either absent or present in only very small numbers.

Fibrin.—The occurrence of fibrin in the urine presupposes the presence of fibrinogen and a fibrinogenic ferment. It is seldom seen. According to Neubauer and Vogel, the fibrin may occur either as coagulated fibrin or in solution. In the former condition it is at times observed in the form of blood coagula, when its significance is essentially the same as that of hematuria in general, although it must be remembered that the usual form of hematuria is not associated with the presence of coagula. Colorless coagula of fibrin are seen in cases of chyluria or diphtheritic inflammation of the urinary passages. On the other hand, urines containing fibrinogenic material in solution are likewise seen but rarely, and are characterized by the fact that fibrinous coagula separate out *on standing*, when they usually cover the bottom of the vessel; but at times they may change the entire bulk of urine into a gelatinous mass. This condition likewise is essentially observed in cases of chyluria, but may possibly also occur in association with nephritis. Lostorfer has reported an instance of this kind, in which fibrinous coagulation took place in the *clear* urine, which contained much albumin, but no blood. Post-mortem chronic inflammatory changes and amyloidosis of the kidney were found, while the urinary passages proper were intact.

Nucleo-albumin.—The question whether or not nucleo-albumin is a normal constituent of the urine is still under dispute. Personal investigations have led me to the conclusion that with complicated methods and large amounts of urine—from 5 to 25 liters—it is always possible to demonstrate its presence both under physiological and pathological conditions. With the usual tests and smaller amounts of urine, however, negative results only are obtained in strictly normal individuals. According to my experience, trichlor-

acetic acid, with which Stewart claims to have obtained positive results in every one of the 150 normal urines which he examined, does not precipitate nucleo-albumin when this is present in normal amounts. *A nucleo-albuminuria recognizable by the available tests does not exist under normal conditions.* Even under pathological conditions nucleo-albumin is by no means always found. Sarzin thus was unable to demonstrate its presence in 200 cases which he examined in Senator's clinic. Citron arrived at similar results, and of several thousand urines which I have examined in this direction positive results were obtained in only a small percentage of cases. It is essentially met with in diseases which directly or indirectly involve the integrity of the epithelial lining of the uriniferous tubules or of the bladder. It has thus been frequently found in cases of acute nephritis and associated with febrile albuminuria, although its presence even then is not constant. In chronic nephritis it is more frequently absent than present. In cases of renal hyperemia and cystitis the results are variable. In 32 icteric urines Obermayer obtained positive results without exception, and it appears that in leukemia nucleo-albumin is also quite constantly present. During the administration of pyrogallol, naphthol, corrosive sublimate, tar preparations, arsenic, etc., as well as in cases of poisoning with anilin and illuminating gas, large amounts of the substance may be found.

According to my experience, nucleo-albumin is frequently obtained in cases of so-called functional albuminuria, and it is not uncommon to find that this is still present when serum albumin and serum globulin can no longer be demonstrated, even with the trichloracetic acid test. Nucleo-albuminuria may thus exist independently of the presence of the more common forms of albumin. This observation has also been made by Strauss, who found nucleo-albumin only in several cases of cystitis, in one case ef chronic interstitial nephritis, and in one case of emphysema pulmonum with renal hyperemia.

The existence of a hematogenic form of nucleo-albuminuria has thus far not been satisfactorily demonstrated. It has been assumed that its presence indicates increased epithelial desquamation in some portion of the urinary tract—in other words, that it is of cellular origin. Matsumoto, however, has shown that even though a urine containing numerous epithelial casts, renal epithelial cells, and leukocytes be allowed to stand for some time, a substance which can be precipitated with acetic acid either does not appear at all or only in very small quantity. He has rendered it very probable that the substance which can be precipitated from pathological urines by means of acetic acid is largely fibrinogen and euglobulin. He adds that nucleo-albumin may be present simultaneously, but in comparison to the other two substances it is of secondary importance and is rarely seen.

Histon and Nucleohiston.—Kolisch and Burian were able to demonstrate the presence of histon in a case of leukemia in which it was constantly present. More recently Krehl and Matthes claim to have isolated the same substance in various febrile diseases, such as acute peritonitis, following appendicitis, in croupous pneumonia, erysipelas, and scarlatina.

It is not clear in what manner the histonuria is produced; so much, however, seems certain, that it is not solely dependent upon increased destruction of leukocytes.

Nucleohiston itself has been found in the urine in a case of pseudoleukemia, by Jolles.

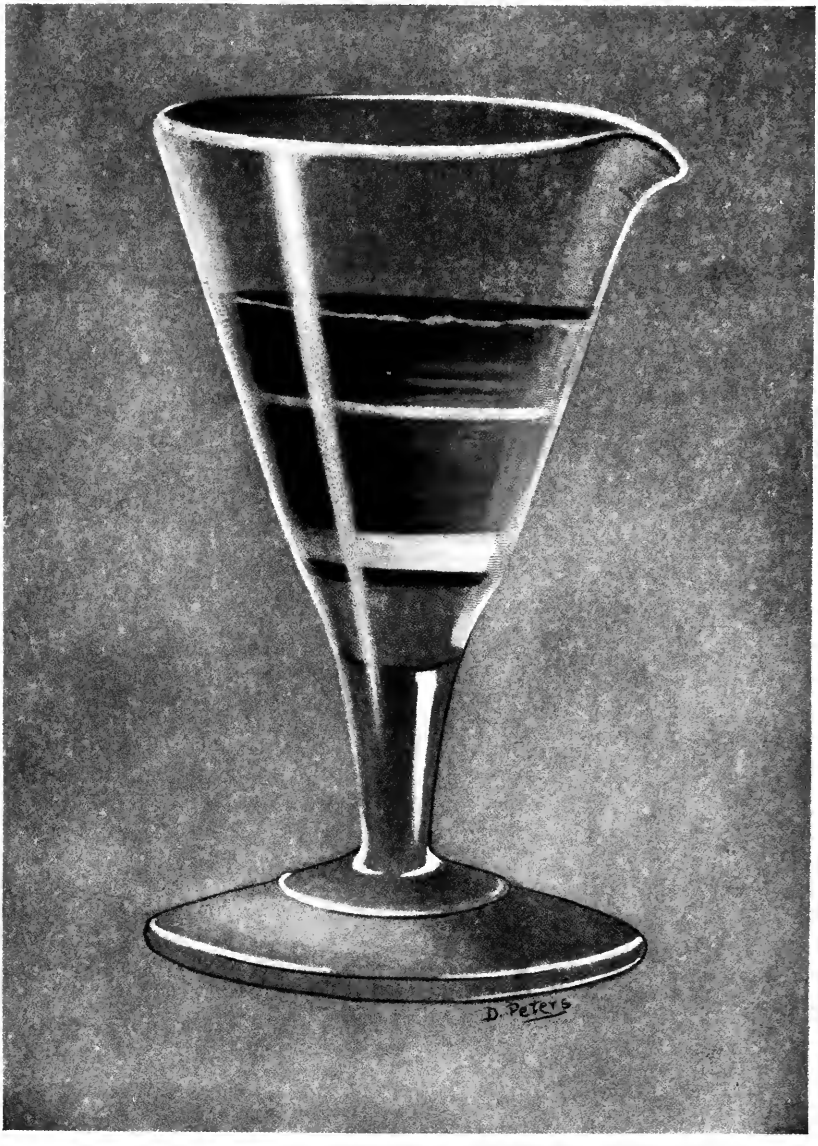
Tests for Albumin.—The recognition of the various albuminous bodies which may occur in the urine is based partly upon their direct precipitation and partly upon color reactions when treated with certain reagents.

The number of tests which have from time to time been sug-suggested is large; many of them after a brief period of use have been discarded as useless or uncertain, while others have been employed only occasionally, and have not received the recognition which they deserve, from the fact that simpler tests exist, that they do not possess sufficient delicacy, or that in some instances it is too great. In the following pages no attempt is made to describe all of these tests, and attention will be directed only to those which are generally used, and which clinical experience has proved to be of value, precedence being given to those which have been longest in use. While some of these are applicable for demonstrating the presence of more than one form of albumin, special tests will also be described whereby the various albumins may be individually recognized.

In every case the urine should be carefully filtered, so as to free it from any morphological elements, etc., present. To this end it is generally sufficient to pass the urine through one or two layers of Swedish filter paper. Frequently, however, a clear specimen cannot be obtained in this manner; it is then advisable to shake the urine with burnt magnesia or talcum or to mix it with scraps of filter paper, when it is filtered as usual.

Tests for Serum Albumin.—*The Nitric Acid Test* (Plate XXIII).—The value of this test, properly applied, cannot be overestimated, as it is not only simple, but yields an amount of information that can otherwise be gained only with difficulty. Usually the student is advised to make use of a test-tube partially filled with urine, along the sides of which concentrated, chemically pure nitric acid is allowed to flow, so as to form a layer at the bottom of the tube, when in the presence of serum albumin a distinct white ring appears at the zone of contact between the two liquids (Heller's test). The pictures thus obtained cannot be compared, however, with those seen when the apparently trivial change is made of using a conical glass of about

PLATE XXIII



Cold Nitric Acid Test.

Albumin ring below; "urate" ring above.

2 ounces' capacity instead of the test-tube. About 20 c.c. of urine are placed in the glass, when 6 to 10 c.c. of nitric acid are added by inclining the glass and allowing the nitric acid to flow down the sides When this is carefully done the nitric acid forms a distinct zone beneath the urine. In the presence of albumin the white ring then appears, and varies in extent and intensity with the amount of albumin present. If now the contents of the glass are allowed to stand undisturbed—and if small amounts are present, the albumin appears on standing for a few minutes—it will be observed that the cloudiness gradually extends upward; and if much albumin is present it may be seen to rise into the supernatant liquid in the form of small, irregular columns. This appearance is possibly referable to the decomposition of uric acid by means of nitric acid, nitrogen, and carbon dioxide being set free, which, rising to the surface in the form of small bubbles, carry the nitric acid upward; coming into contact with albumin in solution, this is then precipitated.

In practically every urine on standing for a few minutes, a fine ring appears in the clear urine above or separated from the albuminous ring by a distinct clear layer of urine (Plate XXIII). This ring has been generally ascribed to the presence of urates, and in certain hospitals of Paris it was long customary to gauge the amount of uric acid by the rapidity with which it forms and its extent. For years I regarded this as an established fact, but I have convinced myself that no relation exists between this phenomenon and the amount of uric acid, as determined by one of the standard methods. Mörner has expressed the opinion that the ring in question is not referable to urates at all, but is of a special albuminous character. Further researches in this direction are needed. Usually the ring is fine and delicate, but at times the substance is present in large amounts and may simulate common albumin, by rapidly extending downward. Its clinical significance is not understood.

Should more than 25 grams of urea be contained in a liter of the urine, an appearance like hoar-frost will be noted on the sides of the glass, which is due to the formation of urea nitrate. Spangles of the same substance appear only in the presence of at least 45 grams; and if 50 grams or more of urea are contained in the liter, a dense mass of urea nitrate may be seen to separate out.

Biliary urine, when treated with nitric acid containing a little nitrous acid, shows the color play referable to the action of nitric acid upon bilirubin. The production of the colors (red, yellow, green, blue, and violet) takes place from above downward, the green color being the most characteristic; in the absence of the latter the presence of biliary pigment may be positively excluded. The presence of albumin does not interfere, as the color play takes place beneath the albuminous disk.

In normal urine a transparent ring is also obtained, presenting a

peach-blossom red; the intensity of this may vary, however, from a faint rose to a pronounced brick color, and is referable to normal urinary pigment. In the presence of urobilin, on the other hand, this ring presents a distinct mahogany color.

Indican is indicated by the appearance of a violet ring situated above that referable to the normal urinary pigment. Its intensity varies with the amount present, from a light blue to a deep indigo.

The albumin ring at the zone of contact of the two fluids may be referable not only to the presence of serum albumin, but also of globulin and albumoses, while a negative reaction indicates the absence of these bodies. Should the precipitate caused by nitric acid consist of albumoses, it will clear up more or less, to reappear on cooling, the fluid at the same time assuming a markedly yellow color. The occurrence of a distinctly yellow color in the urine, moreover, which is only partially cleared upon the application of heat (and be it remembered that a much higher temperature is necessary for the solution of a precipitate referable to albumoses than of one due to urates), will indicate the existence of a mixed albuminuria—*i. e.*, the presence of coagulable albumin and albumoses.

Nitric acid may also cause a precipitation of certain resinous bodies, such as those contained in turpentine, balsam of copaiba and tolu, etc. If any doubt is felt, the mixture should be shaken with alcohol, when the precipitate caused by these substances is at once dissolved.

Nucleo-albumin, which is at times found in the urine, is also precipitated by nitric acid, but need not occupy our attention at this place. From what has been said, it is manifest that the *employment of the nitric acid test in the manner indicated furnishes much valuable information, and the adoption of the method as described not only by hospital students, but by general practitioners as well, cannot be too strongly urged.*

Boiling Test.—A few cubic centimeters of urine are boiled in a test-tube and then treated with a few drops of concentrated nitric acid, no matter whether a precipitate has occurred upon boiling or not. If albumin is present, this will separate out as a flaky precipitate, which consists of serum albumin frequently mixed with serum-globulin. It is true that albuminous urines will generally yield a precipitate on boiling alone; but it must be remembered that unless the reaction is decidedly acid a precipitation of normal calcium phosphate may occur, owing to the fact that the reaction of the urine upon boiling becomes less acid from the escape of carbonic acid held in solution. In urines presenting an alkaline or amphoteric reaction this is very frequently noted, and might give rise to confusion, as the precipitate due to calcium phosphate closely resembles that referable to albumin. In an alkaline medium, moreover, albumin may not be precipitated at all on boiling. Care must hence be taken

to insure a distinctly acid reaction, which is best accomplished by the addition of nitric acid, when a precipitate referable to phosphates is at once dissolved while one due to albumin remains, and may even become more marked. The quantity to be added should usually be equivalent to about 0.05 to 0.1 per cent. of the volume of the urine. Under no condition should the acid be added before boiling, nor should the urine be boiled after its addition, as small amounts of albumin will otherwise be overlooked, owing to the fact that hot nitric acid dissolves the precipitate to a certain degree. If after the addition of the nitric acid the urine turns a distinct yellow, and if then upon cooling a white precipitate appears, the presence of albumoses may be inferred. Uric acid will cause no confusion, as this separates out only upon cooling, and then presents a dark-brown color. As in the case of the nitric acid test, so also here, a precipitation of certain resins is noted at times which may be recognized by their solubility in alcohol. Albumoses are also precipitated upon the application of heat, but such precipitates again dissolve when the temperature approaches the boiling point.

Should acetic acid be used instead of nitric acid, great care must be taken to avoid an excess, as otherwise the albumin will be dissolved. As this danger diminishes the greater the quantity of salts contained in the urine, it is advisable to treat the urine first with a few drops of acetic acid until a distinctly acid reaction is obtained, and then to add one-sixth its volume of a saturated solution of sodium chloride, magnesium sulphate, or sodium sulphate, when upon boiling a precipitation of the albumin will occur. Carried out in this manner, the test is absolutely certain and will demonstrate even minimal amounts of albumin. If an equal volume of a saturated solution of common salt is added to the acidified urine, albumoses are also precipitated, but the precipitate dissolves on boiling.

The Potassium Ferrocyanide Test.—A few cubic centimeters of urine are *strongly* acidified with acetic acid (sp. gr. 1.064) and treated with a few drops of a 10 per cent. solution of potassium ferrocyanide, when, in the presence of but little albumin, a faint turbidity, or, if much albumin is present, a flaky precipitate, is noted, which is best recognized by comparison with a tube containing some of the pure filtered urine, both tubes being held against a black background. Von Jaksch advises the careful addition, by means of a pipette, of a few cubic centimeters of fairly concentrated acetic acid, to which a little potassium ferrocyanide has been added, when the albumin, as in Heller's test, is seen to form a ring at the zone of contact between the two fluids. Instead of potassium ferrocyanide, potassium platinocyanide may also be employed, and has the advantage that the test solution is colorless. Concentrated urines should be previously diluted with water. The presence of albumoses

may be inferred if the precipitate disappears upon boiling, while a partial clearing up indicates the combined presence of albumoses and coagulable albumin.

At times the addition of acetic acid by itself is followed by the appearance of a cloud in the urine, which may be due to urates or to urinary mucin (nucleo-albumin), as already mentioned. In such cases the urine should be refiltered, diluted with water, and the test again applied; nucleo-albumin will dissolve in an excess of the acid.

The Trichloracetic Acid Test.—This test is undoubtedly the most delicate of those so far described, but not so delicate that a trace of albumin or nucleo-albumin can be demonstrated in every urine. An experience based upon the examination of several thousand urines with this reagent warrants my speaking with a certain degree of confidence upon the subject. Very frequently it is possible with this method to demonstrate albumin in urines in which the more common tests yield negative results, but in which tube casts may nevertheless be found upon microscopic examination. The test is applied as follows: By means of a pipette 1 or 2 c.c. of an aqueous solution of the reagent (sp. gr. 1.147) are carried to the bottom of a test-tube containing the carefully filtered urine, so as to form a layer beneath the urine. In the presence of albumin a white ring will be seen to form at the zone of contact between the two fluids, varying in intensity with the amount of albumin present. So far as the test for albumin is concerned, this reagent possesses an advantage over nitric acid in that the colored rings, which are so confusing to the inexperienced, are commonly not observed. Serum albumin, serum globulin, and albumoses are precipitated, the presence of the latter being recognized, as in the previous tests, by the fact that the precipitate disappears upon boiling and reappears on cooling. A cloud, referable to uric acid (?), also appears if this is present in excessive amounts, but disappears upon the application of gentle heat. A previous dilution of the urine, moreover, guards against its occurrence.

Special Test for Serum Albumin.—Should it be desired, for any reason, to demonstrate serum albumin alone, the urine is rendered amphoteric or faintly alkaline with sodium hydrate, and is then saturated with magnesium sulphate in substance, in order to remove any globulin. The filtrate is rendered distinctly acid with acetic acid, when a flaky precipitate, appearing upon boiling, will indicate the presence of serum albumin.

Patein's albumin differs from the common serum albumin in being soluble in acetic acid.

Very often, as in the examination for sugar, it is necessary to remove any coagulable albumin that may be present, to which end the urine is rendered distinctly acid with acetic acid and boiled. An examination of the filtrate with potassium ferrocyanide, if the amount

of acetic acid added was just sufficient, will then yield a negative result.

Quantitative Estimation of Albumin.—For the quantitative estimation of albumin a large number of methods have been devised, which fact in itself is sufficient to indicate that the majority of them, at least, are unsatisfactory.

Old Method by Boiling.—If comparative results only are desired, a definite amount of urine is boiled after acidifying with acetic acid; the albumin is allowed to settle for twenty-four hours. For this purpose Neubauer suggests the use of glass tubes measuring one-half to three-quarters of an inch in diameter, which are closed at the lower end with a cork. Ordinary test-tubes answer perfectly well, but care should be taken that the same quantity of urine is used in each case. The tubes are corked and kept for several days for comparison. The results, of course, express only the relative amount of albumin present, and it should be remembered that the error incurred may amount to as much as 30 or even 50 per cent. of the quantity that is found by gravimetric analysis. This is owing to the fact that sometimes the albumin separates out in large flakes, and at other times in small flakes, and that the degree of precipitation is also influenced by the specific gravity of the supernatant urine.

FIG. 158.—Esbach's albuminimeter.

Esbach's Method.—The reagent is composed of 10 grams of picric acid and 20 grams of citric acid, dissolved in 1000 c.c. of distilled water. Special tubes, termed albuminimeters (Fig. 158), are employed, which bear two marks, one, *U*, indicating the point to which urine must be added, and one, *R*, the point to which the reagent is added. The lower portion of the tube up to *U* bears a scale reading from 1 to 7, corresponding to the amount of albumin pro mille. The tube is filled to *U* with the filtered albuminous urine, and the reagent added until the point *R* is reached. The tube is closed with a stopper, inverted twelve times, and set aside for twenty-four hours.

At the expiration of this time serum albumin, serum globulin, and albumoses, as well as uric acid and kreatinin, will have settled, when the amount pro mille in grams may be read off from the scale. A few precautions must be observed in order to obtain as accurate results as possible. The reaction of the urine should be acid, and if this is not the case acetic acid is added. Its specific gravity should not exceed 1.006 or 1.008, the proper density being obtained by diluting with water. The amount of albumin in the specimen should not exceed 0.4 per cent.; if more be present, as determined by a

preliminary test, the urine should be diluted. Most important, furthermore, is the temperature of the room. This should be 15° C.; variations from this point are apt to give rise to inaccurate results, which, according to Christensen, may amount to 100 per cent. in the case of a deviation of only 5° C. It is thus clear that as generally employed in the clinical laboratory the method will only give approximate results.

Phosphotungstic Acid Method (Tsuchiya's Modification).—The reagent consists of 1.5 grams of phosphotungstic acid, dissolved in 5 c.c. of concentrated hydrochloric acid and 95 c.c. of ethyl alcohol. It is employed in the same manner as the Esbach reagent, but has several important advantages over that method (Mattice). It can thus be relied upon to indicate slight changes in the albumin output, which is not true of the Esbach. The readings are not influenced by changes in temperature to the same extent as with the Esbach. The urine needs no further dilution. The method can be used for large as well as small amounts of albumin. Glucosuria does not interfere with the method. The reagent keeps well and does not stain the hands. Normal urines, according to Mattice, yield a slight precipitate, while this is denied by Tsuchiya.

Gravimetric Method.—If accuracy is required the amount of albumin must be determined gravimetrically as follows: A certain quantity of urine, after having been acidified with an amount of acetic acid sufficient to insure complete precipitation of all albumin, is boiled; the albumin is then filtered off, dried, and weighed. For this purpose, 500 to 1000 c.c. of filtered urine should be available. A specimen of this, if already acid, is placed in a test-tube, in boiling water, until coagulation takes place, when it is further heated over the free flame and filtered. The filtrate is tested with acetic acid and potassium ferrocyanide. Should no albumin be thus demonstrable, the entire amount of urine is treated in the same manner, and requires no further addition of acetic acid. If, however, the test yields a positive result, it is apparent that the urine was not sufficiently acid. The entire volume is then treated with a 30 to 50 per cent. solution of acetic acid, drop by drop, the mixture being thoroughly stirred and specimens tested from time to time, as described. When, finally, the urine remains clear or shows only a faint turbidity, 100 c.c. or less, according to the amount of albumin present, are first heated in boiling water until the albumin begins to separate out in flakes, and then brought to the boiling point over the free flame. The supernatant urine is decanted through a filter, which has been previously dried at 120° to 130° C. and accurately weighed, when the whole amount of the precipitate is brought upon the filter. Any albumin remaining in the beaker is detached from its sides by means of a glass rod tipped with a piece of rubber tubing, and collected by the aid of hot water. The entire precipitate is thoroughly

washed with hot water until the washings no longer become turbid when treated with a drop of nitric acid and silver nitrate; in other words, until the chlorides have been removed. The precipitate is further washed with alcohol and finally with ether to remove any fats that may be present, when it is dried at 120° to 130° C. to a constant weight. If still greater accuracy is required, the dried and weighed precipitate is incinerated to determine the amount of mineral ash in combination with the albumin, which is then deducted from the total weight. The most accurate results are obtained if not more than 0.2 to 0.3 gram of albumin is contained in the amount of urine employed. A smaller quantity than 100 c.c. should hence be used if a previous test with Esbach's albuminimeter shows a higher percentage.

A glass-wool filter insures a more rapid process of drying—twenty-four to thirty hours; but care must be had that this is properly prepared, so as to guard against a loss of the wool while washing.

Test for Serum Globulin and its Quantitative Estimation.—To test for serum-globulin, the urine is rendered alkaline by the addition of ammonium hydrate, any phosphates that may thus be thrown down being filtered off on standing. The urine is then treated with an equal volume of a saturated solution of ammonium sulphate, when the occurrence of a precipitate will indicate the presence of globulins. Ammonium urate may likewise separate out, but this occurs later.

According to Paton, the following test may also be employed: The urine after having been rendered alkaline with sodium hydrate—any phosphates which may separate out are filtered off—is carefully poured down the side of a test-tube containing a saturated solution of sodium sulphate, so as to form a layer above this, when in the presence of serum globulin a white ring will appear at the zone of contact.

If a *quantitative estimation* of the globulin is to be made, the precipitate thus obtained, after about one hour's standing, is collected on a dried and weighed filter, and washed thoroughly with a one-half saturated solution of ammonium sulphate until a specimen of the washings treated with acetic acid and potassium ferrocyanide no longer gives a precipitate. It is then treated as directed in the method employed for the quantitative estimation of serum albumin.

Tests for Albumoses.—A small amount of urine is strongly acidified with acetic acid and treated with an equal volume of a saturated solution of common salt. In the presence of albumoses a precipitate occurs, which dissolves on boiling and reappears on cooling. If serum albumin also be present, which is usually the case, the hot liquid must be filtered. The albumoses are found in the filtrate and appear on cooling. If the *hot* filtrate, moreover, is rendered strongly alkaline with a solution of sodium hydrate, a red color develops upon the addition of a very dilute solution of cupric sulphate (1 to 2 per

cent.), added drop by drop (biuret reaction). On boiling with *Millon's reagent* a red color is also obtained. This reagent is prepared by dissolving 1 part of mercury in 2 parts of nitric acid of a specific gravity of 1.42, and diluting with 2 volumes of distilled water.

Bang's Method.—10 c.c. of urine are heated in a test-tube with 8 grams of finely powdered ammonium sulphate until the salt has been dissolved; the fluid is then boiled for a moment. The hot fluid is centrifugated for one-half to one minute, the supernatant fluid poured off, and the sediment stirred with alcohol in an agate mortar. The alcohol is poured off, and the residue dissolved in a little water; the solution is boiled and filtered, and the filtrate tested with sodium hydrate solution and cupric sulphate as described above. Should the urine be rich in urobilin—*i. e.*, manifesting a well-marked fluorescence with zinc chloride and ammonia—it is best to extract the final aqueous solution with chloroform by shaking, and to pour off the supernatant fluid, when this is tested with cupric sulphate. In this manner it is possible to demonstrate the presence of albumoses in a dilution of 1 in 4000 to 5000. Other constituents of the urine, with the exception of hematoporphyrin, do not interfere with the test. Should hematoporphyrin be present, however, which may be suspected if a red alcoholic extract is obtained, the urine must first be precipitated with barium chloride. The filtrate, which contains the albumoses, is then examined as described.

If a centrifuge is not available, the urine is boiled with the ammonium sulphate, when a portion of the albumoses will remain on the sides of the tube as a sticky mass. This is washed with alcohol, and if necessary with chloroform, dissolved in water, and tested for biuret.

The alcoholic extract may also be used for testing for urobilin; to this end it is only necessary to add a few drops of a solution of zinc chloride, when in the presence of urobilin a beautiful fluorescence will be observed. The test is extremely delicate.

Examination for True Peptone (Polypeptids).—To demonstrate the presence of true peptone (in the sense of Kühne) in the urine, about 300 c.c. of filtered acid urine are saturated on a water bath with ammonium sulphate at a temperature between 60° and 70° C. On cooling, the mixture is filtered, the filtrate is alkalinized with a dilute solution of sodium carbonate, again saturated between 60° and 70° C. with ammonium sulphate, filtered on cooling, the filtrate neutralized with very dilute acetic acid, again saturated with the salt between 40° and 50° C., and finally again filtered on cooling. The final filtrate is diluted with an equal volume of distilled water and treated with a freshly prepared solution of tannic acid, which is added drop by drop, care being taken to avoid an excess. The precipitate is filtered off the next day, dried in the desiccator upon the filter, powdered, and covered in a porcelain crucible with a small amount of baryta water to which a little finely powdered

baryta is added. The mixture is placed on a boiling water bath for three minutes, and after one or two hours it is filtered. If necessary, the solution is decolorized with neutral lead acetate. The biuret test is finally applied, and if positive indicates the presence of peptone in the sense of Kühne.

Tests for Bence Jones' Albumin.—The presence of Bence Jones' albumin is usually discovered on slowly heating the urine to the boiling point. It will then be noted that at a temperature of from 50° to 60° C. a more or less intense, milky turbidity develops, which on subsequent boiling either disappears entirely or partially, and reappears on cooling. The degree to which the urine clears on boiling differs in different cases. As I have just stated, the turbidity may disappear entirely; but, on the other hand, urines are met with in which even a partial clearing can scarcely be made out. This is apparently dependent upon the degree of acidity of the urine, the amount of mineral salts and of urea present, and probably also upon other and still unknown factors; it does not necessarily indicate that common albumin is simultaneously present.

Upon the addition of a drop of nitric acid to a few cubic centimeters of such urine a temporary turbidity develops, which disappears on shaking, but persists if a little more of the acid is added. If now the mixture is heated, the albumin first coagulates to a dense mass; on boiling, this dissolves, and after a while the liquid becomes almost entirely clear, while the turbidity returns, as before, on subsequent cooling. Similar reactions are obtained with all the common reagents for albumin.

The most characteristic reaction is obtained on boiling the urine with ninhydrin (5 c.c. with 0.2 c.c. of a 1 per cent. aqueous solution for one minute), when a deep reddish-purple color is obtained which differs altogether from the blue which results on boiling urine containing the ordinary albumins.[1]

For its complete identification the albumin should be isolated and further examined as follows: Large amounts of urine are precipitated by the addition of one and one-half to two volumes of 96 per cent. alcohol, or by treating with two volumes of a saturated solution of ammonium sulphate. In either event the total amount of albumin is thrown down. This is then washed with alcohol and ether, and dried over sulphuric acid. To purify the substance it is dissolved in boiling water, by the aid of a few drops of a dilute solution of sodium carbonate, and dialyzed to running and then to distilled water until free from mineral salts. It is then reprecipitated with alcohol (if necessary, after the addition of a drop or two of a dilute solution of hydrochloric acid), washed with absolute

[1] This reaction is not due to the Bence-Jones albumin itself, but probably to associated amino acids. The writer regards the test as pathognomonic when obtained in a urine which contains a protein, showing the curious behavior on the application of heat, described in the preceding paragraph.

alcohol and ether, and dried. Thus purified, the albumin is practically insoluble in distilled water or saline solution at ordinary temperature, and only sparingly so at the boiling point. In boiling water, however, it dissolves with comparative ease after the addition of a few drops of sodium carbonate solution. On neutralization no precipitate occurs if a sufficient amount of water is present. If such a neutral solution is heated, no change occurs; but if it is now acidified and a certain amount of salt added, the typical reaction appears on heating, viz., precipitation between 50° to 60° C. (even between 40° and 50° C. if a sufficient amount of salt is present), clearing on boiling, and reprecipitation on cooling.

On digestion with pepsin-hydrochloric acid a proto-albumose is obtained among the early products of digestion, while a hetero-albumose is not formed. (See Bence Jones' Albumin, p. 388).

Test for Nucleo-albumin.—It has been generally supposed that the substance which is precipitated on adding strong acetic acid to certain pathological urines, when diluted two or three times with water, is nucleo-albumin, the precipitate being soluble or largely so in an excess of the reagent. Matsumoto, however, has recently pointed out that the substance which is precipitated in this manner is largely a mixture of fibrinogen (fibrinoglobulin) and euglobulin. Nucleo-albumin may be present at the same time, but it is rare, and its quantity in comparison to the two albumins mentioned insignificant.

To demonstrate the presence of nucleo-albumin, it is necessary to salt out the albumins with ammonium sulphate (half saturation is sufficient), and then to ascertain whether any precipitation occurs within the limits of precipitation of nucleo-albumin. Matsumoto gives these as 0.1 to 0.8 (lower limit) and 1.6 and 2.2 (upper limit). Its limits of precipitation are the lowest of the known albumins.

Whether or not *Ott's test* in the light of this work can still be relied upon as a test for the demonstration of nucleo-albumin may be questioned. It is conducted as follows: A few cubic centimeters of urine are treated with an equal volume of a saturated solution of common salt, when Almén's solution, which consists of 5 grams of tannic acid, 10 c.c. of a 25 per cent. solution of acetic acid, and 240 c.c. of 40 to 50 per cent. alcohol, is slowly added. The development of a precipitate was regarded as evidence of the presence of nucleo-albumin.

In order to remove nucleo-albumin from the urine, this is treated with neutral lead acetate, an excess of the reagent being avoided.

Test for Hemoglobin.—The diagnosis of hemoglobinuria is based upon the demonstration of hemoglobin, viz., methemoglobin, in the urine in solution, in the absence of red corpuscles, or at least in the presence of only a very small number.

Bloody urine is generally turbid, and may vary in color from bright red to almost black.

Oxyhemoglobin, as such, can only be recognized by the spectro-

scope; it gives rise to the appearance of two bands of absorption, situated between D and E, as described in the chapter on the Blood.

The urine to be examined spectroscopically should be rendered feebly acid by means of acetic acid, and placed before the open slit of the spectroscope in a test-tube, beaker, or similar vessel, when the two bands of oxyhemoglobin will be seen, either at once or upon diluting with distilled water. If ammonium sulphide is added, the spectrum of reduced hemoglobin will be obtained. It must be remembered that more commonly the spectrum of methemoglobin is seen in cases of hemoglobinuria.

The following tests, which will also indicate the presence of blood-coloring matter, cannot be employed to decide the nature of the pigment present, as methemoglobin and oxyhemoglobin will both react in the same manner.

Heller's Test.—A small amount of the urine, or still better a portion of the sediment, is made strongly alkaline with sodium hydrate and boiled. On standing, a deposit of basic phosphates forms, which in the presence of blood-coloring matter presents a bright red color. This is referable to the formation of hemochromogen, as may be shown by spectroscopic examination. Thus controlled, the test is extremely sensitive, and still yields a positive result when the chemical test alone leaves one in doubt. The deciding band is the first between D and E. Care should be had, however, that the solution is cold, as otherwise the hemochromogen is transformed into hematin in alkaline solution. At times, when the urine contains a large amount of coloring matter (bile pigment, etc.), it may be difficult to determine the exact color of the sediment. In such cases the subsequent examination with the spectroscope—the lensless instrument of Hering or that of Browning suffices—is invaluable. In the absence of such apparatus the procedure of v. Jaksch may be employed. To this end the phosphatic deposit is filtered off and dissolved in acetic acid, when if blood pigment is present the solution becomes red, the color gradually vanishing upon exposure to the air. The delicacy of the test is such that oxyhemoglobin can still be demonstrated in a dilution of 1 to 4000.

Donogany's Test.—About 10 c.c. of urine are treated with 1 c.c. of a solution of ammonium sulphide and the same amount of pyridin, when in the presence of blood a more or less intense orange color develops, especially if looked at from above, against a white background. In doubtful cases the examination is to be controlled by a spectroscopic examination of the resulting mixture. If blood pigment is present, the spectrum of hemochromogen is obtained. Should the ammonium sulphide and pyridin be old, a green or brown color is imparted to the urine, which changes to yellow upon the addition of ammonium hydrate.

Test for Fibrin.—Fibrin usually occurs in the urine in the form of distinct clots, the nature of which may be determined by thor-

oughly washing with water, when they are dissolved by boiling in a 1 per cent. solution of soda or a 5 per cent. solution of hydrochloric acid. On cooling, this solution is tested as for serum albumin.

Test for Histon.—The urine of twenty-four hours is first examined for albumin, and this removed if present. It is then precipitated with 94 per cent. alcohol, the precipitate washed with hot alcohol and dissolved in boiling water. Upon cooling, the solution thus obtained is acidified with hydrochloric acid and allowed to stand for several hours. During this time a cloudiness, referable to a large extent to uric acid, develops, which is filtered off, and the filtrate is precipitated with ammonia. The precipitate is collected on a small filter and washed with ammoniacal water until the washings no longer give the biuret reaction. It is then dissolved in dilute acetic acid and the solution tested with the biuret test; if this yields a positive result, and if coagulation occurs upon the application of heat, the coagulum being soluble in mineral acids, the presence of histon may be inferred.

CARBOHYDRATES

Glucose.—Through the researches of Wedenski, v. Udrańszky, and others, we know that traces of glucose may be encountered in the urine under strictly normal conditions. The amount, however, is extremely small, and special methods are necessary in order to demonstrate its presence. With the usual clinical tests normal urine is apparently free from sugar unless unduly large amounts have recently been ingested. In that event a certain amount of glucose is eliminated in the urine, constituting the so-called *digestive glucosuria* of Claude Bernard.

The normal limit to the assimilation of glucose on the part of the body economy is subject to considerable variation. Some observers thus report that the ingestion of such large amounts as 200 and 250 grams does not lead to glucosuria, while others have found sugar in the urine after the administration of 100 grams. In view of the possible reaction existing between diabetes and a lowered limit to the assimilation of glucose in apparently normal individuals, or at least in persons in whose urine glucose cannot be constantly demonstrated, this question has created much interest within the last few years and has called forth a large amount of work. The majority of investigators are now in accord in regarding as abnormal a glucosuria that follows the ingestion of 100 grams of chemically pure glucose.

The method usually employed in order to ascertain the power of assimilation for glucose is the following:

The patient receives 100 grams of glucose, in substance, dissolved in 500 c.c. of water, on an empty stomach, and is instructed to pass his water hourly during the following four or five hours.

During this time no food is to be taken. The individual specimens, as well as the urine which has been passed during the night, are then tested with Trommer's and Nylander's tests, with the fermentation test, and with phenylhydrazin. A positive result, however, is recorded only when sugar can be demonstrated with the fermentation test.

Cane sugar and larger amounts of glucose have also been used; but it is better, on the whole, as Strauss has pointed out, to give glucose, and not to exceed the dose of 100 grams.

Especially interesting are the results which have been obtained in various diseases of the liver. Bierens de Haën thus reports that of 29 cases of various hepatic diseases, he found sugar in 18 after the administration of 150 grams of cane sugar; and v. Jaksch claims to have obtained positive results in 15 cases of phosphorus poisoning out of 43. Strauss, on the other hand, states that he found sugar in only 2 of his 38 cases, and has collected 107 additional cases from the literature, in only 14 of which could sugar be demonstrated. If we add these together, we have 145 cases of various hepatic diseases, with negative results in 88.9 per cent. Referring to the contradictory results obtained, Strauss points out that these may have been accidental in part, but that the interpretation which has been offered by v. Jaksch and de Haën may not have been correct. It is thus possible that in his case of phosphorus poisoning other factors besides the changes in the liver, such as the action of the poison upon the nervous system, etc., played a *role*, as a digestive glucosuria may also occur in connection with other forms of intoxication, as in fevers, following the administration of large doses of diuretin, in acute alcoholism, etc., in which the liver is not the only organ that is involved. Strauss further shows that great care must be exercised in the selection of the material for such investigations, and believes that errors referable to this source may have been incurred by Bierens de Haën. He thus cites 2 cases of hypertrophic cirrhosis, associated with delirium tremens, in which small amounts of sugar could be demonstrated in the urine a few days after recovery from the delirium, while shortly after negative results only could be obtained. The lowering effect of alcoholism upon the limit to the assimilation of glucose is a well-known phenomenon, and it would be erroneous to conclude that because alcoholism may call forth organic changes in the liver the digestive glucosuria in such cases must be referable to such alterations. Without entering further into the question at this place, it appears that diseases of the liver *per se* do not materially lessen the power of assimilation for glucose, and that other forces are at the disposal of the body to supply the glycogen-forming or retaining power of the liver when this becomes insufficient, and that these also may be at fault when a digestive glucosuria is observed in association with hepatic disorders.

The association of digestive glucosuria with various diseases of the nervous system has been studied by v. Jaksch, Strümpell, H. Strauss, von Oordt, Geelvink, and Arndt. From the work of these investigators it appears that digestive glucosuria is rarely seen in spinal diseases, and is decidedly more common in functional diseases of the central nervous system than in organic affections. Of 30 cases of tabes examined by Strauss, digestive glucosuria resulted in only 1 after the administration of 100 grams of glucose, and in that case a family history of diabetes existed. In 16 further cases examined by J. Strauss negative results were obtained. In the neuroses a positive result was noted in 42 out of 210 cases which I have been able to collect from the literature. Most frequently it is met with in the traumatic neuroses, in which Strauss observed the phenomenon in 37.5 per cent. of his 40 cases; while in the non-traumatic forms only 14.4 per cent. were insufficient in this respect. Of the organic diseases of the central nervous system, it appears that diffuse cerebral lesions referable to alcohol and syphilis are more likely to give rise to this form of glucosuria than the more localized lesions. In general paresis digestive glucosuria is thus not uncommon (H. Strauss, Arndt). but it is only possible to draw definite deductions from the study of a large amount of clinical material. Small series like that of J. Strauss do not give a proper idea of actual conditions, as he, for example, obtained negative results in all of 10 cases.

In his examination of 5 cases of idiocy and 23 cases of imbecility, J. Strauss obtained positive results in only 2 of the imbeciles after the administration of 100 grams of glucose; in both of the positive cases the glucosuria was transitory and associated with the existence of nervous excitability. Bergenthal observed alimentary glucosuria in 6 cases out of 20.

In Basedow's disease digestive glucosuria has also been noted in a large number of cases by Chvostek, Kraus and Ludwig, Strauss, Goldschmidt, and Stern. Especially interesting in this connection is the fact that digestive glucosuria may be induced by the administration of thyroid extract, viz., thyroidin or iodothyrin in apparently normal persons. Bettmann thus noted glucosuria after the ingestion of 100 grams of glucose in 12 of 25 healthy individuals who had been treated for a week with the products in question.

A digestive glucosuria is further observed in numerous febrile diseases, such as pneumonia, typhoid fever, acute articular rheumatism, scarlatina, tonsillitis, etc. The amount of sugar usually found varies from 0.5 to 3 per cent.; larger amounts may, however, also be encountered, and 1 case is on record in which 8 per cent. was present.

Very common also, as I have indicated, is the digestive glucosuria of alcoholics, and there can be little doubt that the habitual ingestion of large quantities of beer and spirits is apt in the course of time to

lead to a more than temporary insufficiency of the carbohydrate metabolism. In the course of his investigations in this direction, Krehl found among the Jena students that the proportion of those in whose urine sugar appeared apparently varied with the different kinds of beer, but was much greater after morning drinking. Of 14 who drank bock or export beer in the morning, 5 had glucosuria. After the evening drinking, amounting in 1 case to seven liters, of 19 only 1 had sugar in the urine, and with Bavarian beer 1 of 11.

Of diseases of the skin, digestive glucosuria is notably associated with psoriasis; and it is interesting to note that the same disease is not infrequently seen in diabetic patients. Gross thus records 5 cases, in 4 of which the psoriasis had existed for many years before the appearance of diabetic symptoms. Similar instances are recorded by Strauss, Grube, Polotebuoff, Nielssen, Schütz and others. Nagelschmidt was able to produce glucosuria by the ingestion of 100 grams of glucose in 8 cases out of 25.

During pregnancy digestive glucosuria is also frequently observed, and is by some regarded as a fairly constant symptom and of diagnostic importance. After delivery the power of assimilation for glucose no longer appears to be subnormal. The milder form of glucosuria in pregnancy is during the last week or two accompanied by lactosuria.

A digestive glucosuria has further been observed in acute and chronic lead poisoning, poisoning with nitrobenzol, anilin dyes, opium, atropin, and carbon monoxide; in the early stages (the first twelve days) of acute phosphorus poisoning, etc. In these conditions, however, the phenomenon has received little attention.

In patients afflicted with disease of the heart, liver, and kidneys Gobbi observed a digestive glucosuria, after the ingestion of from 100 to 200 grams of glucose, if diuretin was at the same time administered.

Very important is the fact that in diabetes mellitus the sugar may at times disappear from the urine, while its elimination is replaced by an excessive excretion of uric acid or phosphates. (See Diabetes.)

The digestive glucosuria to which reference has been made in the preceding pages is generally spoken of as the *digestive glucosuria e saccharo*. Similar results have been obtained after the administration of starches in excess, viz., 150 to 200 grams. But while a digestive glucosuria e saccharo is regarded only as a possible indication of a pathological alteration of the carbohydrate metabolism, it is generally thought that every *glucosuria ex amylo* is indicative of a definite disturbance in the sense of diabetes, unless special factors, such as an increase of the surrounding temperature, diminished radiation of heat, or complete lack of muscular activity, are active. Strauss, however, has shown that in cases in which a somewhat more than temporary predisposition toward glucosuria e sac-

charo exists, as in alcoholics, for example, a coincident tendency toward glucosuria ex amylo may likewise be demonstrated. As a result of his experiments he concludes that the difference between the digestive glucosuria e saccharo and glucosuria ex amylo is essentially a question of degree. *Cæteris paribus*, it appears that harmful influences of a slight character lead to glucosuria e saccharo, while grave insults call forth glucosuria ex amylo. It results practically that the prognosis in those cases in which digestive glucosuria follows a temporary insult is far better than when the carbohydrate metabolism is permanently damaged, and especially when a glucosuria ex amylo accompanies a glucosuria e saccharo. In the first instance it is scarcely likely that true diabetes will develop in the course of time, while in the latter this is at least possible.

Aside from the digestive form of glucosuria which has just been considered, and which is produced artificially, an idiopathic transitory form is also known to occur. A *transitory glucosuria*, apparently of central origin, is thus noted in connection with lesions affecting the central as well as the peripheral nervous system, such as tumors and hemorrhages at the base of the brain, lesions of the floor of the fourth ventricle, cerebral and spinal meningitis, concussion of the brain, fracture of the cervical vertebræ, tetanus, sciatica; following epileptic, hystero-epileptic, and apoplectic seizures, mental shock produced by railroad accidents (traumatic neuroses), etc.; mental strain and worry, fatigue, and anxiety. Glucosuria following epileptic and apoplectic attacks, however, does not appear to be so common as is generally believed.

In Basedow's disease transitory glucosuria may also occur, and it is well established that a relation may exist between the disease in question and diabetes mellitus.

Siegmund noted a transitory glucosuria in 52.38 per cent. of general paretics, in 7.4 per cent. of epileptics, and in 3.77 per cent. of dementia cases, while it was not observed in other mental diseases. In reference to the postepileptic glucosuria which has been noted by some of the older observers more especially, an analysis of their work has led me to the conclusion that their inferences were scarcely justifiable, as a wholly satisfactory proof of the presence of sugar has not been furnished.

In cases of cholelithiasis, contrary to what has been maintained by one or two observers, glucosuria is unusual.

It is well known that Claude Bernard experimentally produced a transitory glucosuria by puncturing a certain spot in the floor of the fourth ventricle, the supposed origin of the hepatic vasomotor nerves, and it is not improbable that this neurotic form of glucosuria is due to some direct or reflex influence affecting that portion of the medulla.

The transitory glucosuria occasionally observed in acute febrile

diseases, such as typhoid fever, scarlatina, measles, cholera, diphtheria, influenza, and especially malaria, particularly during convalescence, may possibly be referable to the action of specific toxins upon this centre. Seegen reports 5 cases of malaria with "diabetes" in which *both conditions* disappeared under the administration of quinine.

A glucosuria of toxic origin has been noted in cases of poisoning with curare, chloral hydrate, sulphuric acid, arsenic, alcohol, carbon monoxide, morphine, etc., and even after simple infusion of normal salt solution into the blood. Phloridzin, a glucoside obtained from the bark of the root of the apple tree, will likewise cause sugar to appear in the urine. The glucosuria thus produced is, however, only temporary, and ceases upon withdrawal of the drug. Of interest is the glucosuria which occasionally follows the administration of thyroid extract or of iodothyrin, as there is evidence to show that in such cases a special predisposition to glucosuria exists. When carried to an extreme degree true diabetes may develop, which subsequently cannot be arrested by withdrawal of the substance.

A persistent form of glucosuria is noted in connection with certain lesions of the brain, especially those affecting the floor of the fourth ventricle. It is also observed after removal of the thyroid gland, and in cases in which thyroid extract has been administered in unduly large amount.

A continuous elimination of sugar, however, is noted principally in the complex of symptoms to which the term *diabetes mellitus* has been applied (which see).

A *digestive galactosuria* (an elimination of more than 2 grams, following the administration of 40 grams) occasionally occurs in diseases of the liver, but is on the whole unusual and cannot be used for diagnostic purposes. It seems to be more common in febrile jaundice, however, than in other conditions.

Tests for Sugar.—Trommer's Test.—A few cubic centimeters of urine are strongly alkalinized with sodium hydrate solution, and treated with a 5 per cent. solution of cupric sulphate, added drop by drop, until the cupric oxide formed is no longer dissolved. The mixture is carefully heated, when in the presence of sugar a yellow precipitate of cuprous hydroxide is formed, which gradually settles to the bottom as a sediment of red cuprous oxide. Albuminous urines must first be freed of albumin by coagulation.

It is important to note that while sugar, unless present in mere traces, can readily be detected in this manner, other substances are or may be present in the urine, such as uric acid, kreatin and kreatinin, allantoin, nucleo-albumin, milk sugar, pyrocatechin, hydroquinone, and bile pigment, which likewise reduce cupric oxide. Following the ingestion of benzoic acid, salicylic acid, glycerin, chloral, sulphonal, etc., reducing substances also appear. These may gen-

erally be disregarded, it is true, if care is taken *not to boil* the urine after the addition of the cupric sulphate, as the precipitation of cuprous oxide in the presence of sugar takes place before this point is reached. Unfortunately, however, the test when thus applied yields negative results, or results which are doubtful, if traces only are present, so that it cannot be utilized, as a rule, in the study of transitory or digestive glucosuria.

Fehling's Test.—This is a modification of the test just described, and can be recommended only with the same restrictions.

Two solutions are employed, which must be kept in separate bottles, the one containing 34.64 grams of crystallized cupric sulphate, dissolved in 500 c.c. of distilled water, and the other 173 grams of potassium and sodium tartrate and 50 to 60 grams of potassium hydrate, dissolved in an equal volume of water. Equal parts of the two solutions, mixed in a test-tube and diluted with four times as much water, are boiled, when a small amount of urine is added. In the presence of sugar a precipitate of the yellow hydroxide of copper or of red cuprous oxide will be produced; but *care should be taken only to warm, and not to boil the solution after the addition of the urine.*

Not infrequently it will be observed that upon standing, when no precipitation has occurred previously, the blue color of the mixture changes to an emerald green, while the solution at the same time becomes turbid. Such a phenomenon should not be referred to the presence of sugar, as it is in all probability due to the action of other reducing substances, such as those mentioned above.

Benedict's Test.—Benedict has pointed out that the reduction of the copper solution is not due to the sugar itself, but to a derivative of the same which is formed as a result of the action of the alkali upon the sugar molecule. As the alkali destroys the reducing action of this sugar derivative after a relatively short time, it is clear that small amounts of sugar might be overlooked. He has pointed out, moreover, that many non-specific reductions of Fehling's solution are due to the action of the hydroxide upon these bodies and that such pseudo-reactions may be avoided, while the test for sugar is rendered more sensitive if alkaline carbonate is substituted for the hydrate.

A staple reagent is secured if sodium or potassium citrate is substituted for the Rochelle salt of Fehling's solution. Benedict's reagent is prepared as follows: 173 gm. of sodium or potassium citrate together with 200 gm. of crystallized sodium carbonate (or half of this quantity of the anhydrous salt) are dissolved together in about 700 c.c. of water, by the aid of heat. The mixture is then poured (through a filter, if necessary) into a large beaker or casserole, and the copper sulphate (dissolved in about 100 c.c. of hot water) added, while stirring. On cooling, water is finally added to the 1000 c.c. mark. The reagent keeps indefinitely.

To test for sugar 5 c.c. of the reagent are placed in a test-tube; 8 to 10 drops of urine are added and the mixture boiled for one or two minutes. If no sugar be present the liquid either remains clear altogether or becomes slightly turbid (on cooling) owing to a precipitation of urates. If the amount of sugar be small (less than 0.3 per cent.) a precipitation of hydrated suboxide of copper occurs only on cooling, while if a larger amount be present the entire bulk of fluid becomes turbid. But whereas with Fehling's solution, owing to the dehydrating action of the strong alkaline hydrate, the red suboxide of copper is apt to separate out; with Benedict's reagent the yellow or greenish-yellow hydrate of the suboxide is precipitated.

Böttger's Test with Nylander's Modification.—A few cubic centimeters of urine are treated with *Almén's solution* in the proportion of 11 to 1. This is prepared by dissolving 4 grams of potassium and sodium tartrate, 2 grams of bismuth subnitrate, and 10 grams of sodium hydrate in 90 c.c. of water, heating the solution to the boiling point, and filtering upon cooling, when it should be kept in a colored glass bottle. The mixture of urine and Almén's fluid is thoroughly boiled, when in the presence of sugar a grayish, dark-brown, and finally a black precipitate, consisting of bismuthous oxide or of metallic bismuth, is obtained. Albumin, if present, must first be removed, as, owing to the sulphur contained in the albuminous molecule, alkaline sulphides would be formed upon boiling, and, acting upon the bismuth, give rise to the formation of black bismuth sulphide, which might be mistaken for metallic bismuth. Rhubarb pigment, as well as melanin and melanogen (which see), and free hydrogen sulphide must also be absent, as misleading results will otherwise be obtained.

Nylander's test, as well as that of Trommer and Fehling, is, however, not without objections, as a partial reduction of the bismuth subnitrate may be produced by other substances, such as kairin, tincture of eucalpytus, turpentine, and large doses of quinine.

Fermentation Test.—This is based upon the fermentative decomposition of sugar with the formation of carbon dioxide and alcohol. It should be resorted to in all doubtful cases. The test is now almost always carried out in special fermentation tubes, such as those of Einhorn (Fig. 159) and Lohnstein (Fig. 160). To this end a small piece of compressed yeast (a fair sized pill) is broken up in a test-tubeful of urine. It is better to do this with a glass rod than by shaking. The fermentation tube is filled with this mixture, care being taken that no bubbles of air remain at the top; the tube is then kept at a temperature of 30° to 38° C. for twenty to twenty-two hours. At the end of this time it is inspected to see whether any gas has been formed. In the case of sugar urines it can readily be proved that the gas is carbon dioxide by introducing some caustic alkali into the tube, when the gas is absorbed.

In every case it is necessary to make a control test with normal urine of approximately the same concentration, as the common commercial yeast always develops a little carbon dioxide by itself. A little bubble is thus usually seen. But the same may occur from the liberation of gas which may be present in absorption, when the tube is kept at the temperature indicated. Unless traces of sugar (less than $\frac{1}{10}$ per cent.) be present no difficulty will result from this fact, as the volume of gas in the sugar urine will exceed that of the control. But when smaller quantities are present some doubt may arise. In that case an attempt must be made to increase the volume of gas by heating, when the sugar urine, owing to the presence of carbon dioxide, will show a larger bubble of gas than the control. This may be done on a boiling-water bath by placing both fermentation tubes, closed off by means of mercury, in a large beaker filled with water so that the tops of the tubes are just covered, and heating for half an hour.

FIG. 159.—Einhorn's saccharimeter.

If a positive result is obtained with the fermentation test the presence of a fermentable sugar is proved; the question whether this is dextrose or levulose, which alone enter into consideration in disease, is practically unimportant. Should blood, pus, albumin, or albumose be present, these should first be removed.

Rarely it will happen that the urine undergoes ammoniacal decomposition in the tubes; if it does occur the examination should be repeated.

Phenylhydrazin Test.—As originally proposed by v. Jaksch, the test is conducted as follows: 6 to 8 c.c. of urine are treated with 0.4 to 0.5 gram of phenylhydrazin hydrochlorate and 1 gram of sodium acetate, and warmed until the salts have been dissolved, a little water being added if necessary. The tube is placed in boiling water for twenty to thirty minutes, and then transferred to a beaker filled with cold water. If sugar is present in moderate amounts, a bright yellow, crystalline deposit will at once be thrown down and partly adhere to the sides of the tube. But even in toe presence of mere traces a careful microscopic examination will reveal the presence of

crystals of phenylglucosazone. These are seen singly or arranged in bundles and sheaves composed of delicate, bright-yellow needles which are insoluble in water.

Still more convenient is the following modification of the test, as suggested by Cipollina: 5 drops of pure phenylhydrazin, 0.5 c.c. of glacial acetic acid, or 1 c.c. of 50 per cent. acetic acid are placed in a test-tube together with 4 c.c. of urine. The mixture is boiled for about one minute over a small flame, while shaking so as to avoid bumping as much as possible; 4 or 5 drops of sodium hydrate solution (specific gravity 1.16) are added, but the solution must remain acid; the boiling is continued for a few seconds and the mixture then allowed to cool. The rapidity with which the glucosazone crystals separate out depends somewhat upon the specific gravity of the urine. If this is low they form in a few minutes, even though the amount of sugar does not exceed 0.05 per cent. If, on the other hand, the specific gravity is high, yellow balls and thornapple forms result, while typical rosettes develop only after twenty to thirty minutes, and at times one is even then left in doubt as to the result. If the urine contains more than 0.2 per cent. of sugar, however, even though the specific gravity be high, the formation of typical crystals occurs within a few minutes. If with this modification no crystals are obtained at the expiration of an hour, we may infer that no sugar is present.

This test, properly applied, is undoubtedly not only the most delicate, but at the same time the most reliable, as no other substances which may be present in the urine, excepting maltose and certain pentoses, will give rise to the formation of an osazone. Hence, whenever doubt is felt as to the nature of a substance reacting in a positive manner with the reagents described above, recourse should be had to this test. It has been stated that maltose forms an exception; this, however, will never become embarrassing, as the microscopic appearance of the maltosazone crystals differs from that of the phenylglucosazone. The melting point of phenylglucosazone (205° C.), moreover, is about 15° higher than that of the maltosazone, viz., 190° to 191° C. To determine this point, it is necessary to filter off the osazone, and, after washing with water, to dissolve it upon a filter by means of a little hot alcohol. From this alcoholic solution it is reprecipitated by water, when it may be collected and dried over sulphuric acid. The melting point is then determined according to the usual methods.

The pentosazones also can be readily distinguished from glucosazone by their melting points (which see).

The amount of lactose which may be found in the urine is far too small to give rise to the formation of an osazone when the test is directly applied to the urine.

With the conjugate glucuronates phenylhydrazin also combines to

form crystalline compounds, but these may likewise be distinguished by their melting points and the form of the crystals. Such compounds, moreover, are usually not present in amounts sufficient to give rise to confusion. (See Glucuronic Acid.)

Polarimetric Test.—Glucose turns the plane of polarized light to the right, but the same may be said of maltose, the degree of polarization of which is even more marked, so that it may be impossible to state in a given case whether such rotation is referable to a large quantity of glucose or to a smaller quantity of maltose. The latter substance, however, occurs in the urine but rarely, and may be recognized not only by the microscopic appearance of its osazone, but also by the fact that its power of reduction is increased in the presence of sulphuric acid and by the application of heat.

An error which may further arise with the employment of the polarimetric method is referable to the fact that if glucose is present in only small amounts, while the urine contains large quantities of β-oxybutyric acid, the latter turning the plane of polarized light to the left, it may happen that the rotation in this direction will neutralize or even counterbalance any rotation to the right, which may be due to glucose. In such cases, however, the urine will react in a positive manner with the other reagents described, and the fermented urine will, moreover, turn the plane of polarization still more strongly to the left, indicating the presence of a dextrorotatory substance, and in all probability of glucose.

The delicacy of this method varies with the instrument employed; the figures given below were obtained with the apparatus of Lippich, which yields the best results. (For a description of this method see the Quantitative Estimation of Sugar by Means of the Polarimeter.)

Table Showing the Delicacy of the Tests Described

Trommer's test	0.0025	per cent.
Fehling's test	0.0008	"
Nylander's test	0.025	"
Fermentation test	0.1 to 0.05	"
Phenylhydrazin test	0.025 to 0.5	"
Polarimetric test	0.025 to 0.05	"

Clinically, it is unimportant to search for minute traces of sugar, such as may be found in every normal urine, and the reader is referred to special works on physiological chemistry for a consideration of the methods generally employed. (See method of Baumann and v. Udranszky.)

Quantitative Estimation of Sugar.—The methods used in the quantitative estimation of sugar are essentially based upon the qualitative tests described.

Fehling's Method.—The Fehling solution (see above: qualitative tests) must be accurately standardized as follows: 0.2375 gram of pure crystallized cane sugar, dried at 100° C., is dissolved in 40 c.c.

of distilled water, to which 22 drops of a 10 per cent. solution of sulphuric acid have been added. This solution is kept on the boiling-water bath for an hour, when it is allowed to cool and diluted to 100 c.c. with distilled water; 20 c.c. of this solution will then contain exactly 0.05 gram of glucose, corresponding to 10 c.c. of Fehling's solution, if this is of the required strength. If too strong, so that 21 c.c. of the sugar solution, for example, are required to obtain a complete reduction of the copper, the strength of Fehling's solution may be determined according to the equation, $20 : 0.05 :: 21 : x$; and $x = 0.0525$. If too weak, on the other hand, so that 19 c.c., for example, are required, its strength is similarly determined—$20 : 0.05 : : 19 : x$; and $x = 0.0475$.

TABLE SHOWING THE BEHAVIOR OF THE VARIOUS FORMS OF SUGAR WHICH MAY OCCUR IN THE URINE TOWARD THE TESTS DESCRIBED

	Trommer's, viz., Fehling's test.	Nylander's test.	Fermentation test.	Phenylhydrazin test.	Polarimetric test.
Glucose.	Positive reaction.	Positive reaction.	Positive reaction.	Positive reaction, melting point 205° C.	Rotation toward the right.
Levulose.	Positive reaction.	Positive reaction.	Positive reaction.	Same osazone obtained as with glucose, only more rapidly.	Rotation toward the left.
Maltose.	Positive reaction.	Positive reaction.	Positive reaction.	A maltosazone is formed; melting point 190° to 191° C.	Rotation toward the right.
Lactose.	Positive reaction.	Positive reaction.	No reaction or only a very faint one.	No reaction in the concentration in which it may occur in the urine; melting point 200° C.	Rotation toward the right; increased by boiling with a 2.5 per cent solution of sulphuric acid.
Laiose.	Positive reaction on boiling only; 1.2 to 1.8 per cent. more is obtained than by the polarimeter.	Positive reaction.	No reaction.	With phenlyhydrazin a yellowish brown, noncrystallizable oil is obtained.	No reaction, or rotation toward the left.

If the solution is of the theoretically required strength 10 c.c. will correspond to 0.05 gram of glucose.

If then urine is added to this quantity until complete reduction has taken place, the amount of sugar in a given specimen of urine can be calculated according to the following equation:

$$y : 0.05 : : 100 : x; \text{ and } x = \frac{5}{y},$$

in which y indicates the number of cubic centimeters of urine required to reduce the 10 c.c. of Fehling's solution, and x the amount of sugar contained in 100 c.c. of urine.

As the best results are obtained if from 5 to 10 c.c. of urine are

used in one titration, it is often necessary to dilute the urine to this end; in the determination of this point the specific gravity may serve as a guide. As a general rule, urines of a specific gravity of 1.030 should be diluted five times, and if the density is still higher ten times. Albumin, if present, must first be removed by boiling.

Ten c.c. of Fehling's solution diluted with 40 c.c. of water are placed in a porcelain dish and boiled. While boiling, the diluted urine is added from a burette, 0.5 c.c. at a time, when, as a rule, the precipitated cuprous oxide will settle, so that the white sides of the dish may be seen through the blue field. In my experience it is very helpful to boil the mixture for a few moments after every addition of urine and to stir thoroughly each time with a rubber-tipped rod. In this way the precipitate is prevented from forming a coating on the vessel and settles down more readily. As the end point is reached every trace of blue has disappeared and the liquid has a faint yellowish tinge owing to beginning caramelization of the excess of sugar by the caustic alkali.

If any doubt should arise whether the end point has been reached, tiny droplets of the mixture should be placed upon ferrocyanide paper (prepared by soaking filter paper in a moderately dilute solution of potassium ferrocyanide). If unreduced copper is still present a brown color results. The result is regarded as positive only if the brown develops at once. If it occurs only after several seconds, the final point has been reached or passed.

Prolonged boiling always brings some copper into solution again. It is hence advisable to make two examinations always, the one approximately only, and the second as the final one.

The calculation is then made as indicated above.

Example.—The volume of urine for twenty-four hours was 4000 c.c. It was diluted five times; 6 c.c. of the diluted urine brought about the complete reduction of 10 c.c. of Fehling's solution; the 6 c.c. hence contained 0.05 gram of sugar; 100 c.c., accordingly, contained 0.833 gram. As the urine had been diluted five times, this figure must be multiplied by 5 = 4.165, which is the percentage for the native urine. The amount for the twenty-four hours was hence $4.165 \times 40 = 166.6$ grams.

Benedict's Method.—With this method the reduced copper is not precipitated as suboxide, but as cuprous sulphocyanate which is snow-white in color, so that the end point at which the blue color of the fluid disappears is easily recognized.

The reagent is prepared as follows: 125 gm. of potassium sulphocyanate, 200 gm. of crystallized sodium carbonate (or one-half of this quantity of the anhydrous salt) and 200 gm. of sodium or potassium citrate are dissolved, by the aid of heat, in about 800 c.c. of water (filtering if necessary). To this solution 18 gm. of pure crystallized copper sulphate (accurately weighed), dissolved in about

100 c.c. of distilled water are slowly added, while stirring; then 5 c.c. of a 5 per cent. solution of potassium ferrocyanide, and on cooling, water to the 1000 c.c. mark. The reagent is stable. 25 c.c. of this reagent will be reduced by 0.05 gm. of glucose.

The actual titration is carried on as follows: 25 c.c. of the above reagent are placed in an evaporating dish together with from 10 to 20 gm. of crystallized sodium carbonate and a pinch of pumice stone or talcum powder (to prevent bumping). The mixture is brought to the boiling point, when the urine (diluted if necessary) is run in, rather rapidly from a burette, until every trace of blue has disappeared, the boiling being continued all the time. Water may be added if the fluid becomes too concentrated. The calculation then is a simple matter, bearing in mind the titer of the reagent and the degree of dilution of the urine (see p. 416).

Chloroform, if present as preservative, must first be removed by boiling.

Gerrard and Allan's Method (Modified by Rudisch and Celler).—To obviate some of the difficulties which attach to Fehling's method, Rudisch and Celler have suggested the following modification of Gerrard and Allan's method:

"To four parts by volume of a 50 per cent. solution of potassium sulphocyanate, chemically pure, is added one part by volume of a mixture of equal parts of Fehling's copper sulphate and alkaline solutions. 25 c.c. of this solution are placed in a porcelain dish, and the urine to be tested added drop by drop from a burette until the blue color of the copper entirely disappears. Throughout the titration the solution should be slowly boiled and constantly stirred with a glass rod. The end reaction is extremely sharp, the fluid becoming colorless or assuming a faint-yellow tinge. The advantages of this method are: (1) only one titration is necessary, as potassium sulphocyanate does not decolorize the copper solution; (2) potassium sulphocyanate is not poisonous; (3) as the mixture is stable a considerable quantity may be made to be kept as 'stock.' Such a 'stock' solution was found to be unaffected after four months' exposure to heat and sunlight.

"With aqueous solutions of glucose ranging from 0.25 to 6 per cent. the results obtained with this method and with the polariscope are identical. With diabetic urines, however, variations of from 0.03 to 0.25 per cent. are occasionally found—differences that are too small to be of clinical significance. These variations are explicable on two grounds: First, substances other than glucose (creatinin, uric acid, glucuronic acid) reduce copper and give too high a reading with Fehling's solution; secondly, levorotating substances (albumin, levulose, β-oxybutyric acid) may co-exist with the glucose in the urine, giving too low a percentage with the polariscope. To estimate properly the quantity of dextrose in any given

specimen, therefore, it is necessary to make determinations both with the copper solution and with the polariscope. Should the former indicate a higher percentage than the latter, levulose should be suspected and tested for with the Seliwanoff resorcin-hydrochloric acid method. In the absence of levulose the most probable disturbing factor is β-oxybutyric acid, as albumin and other levorotators are precipitated when the urine is cleared with lead acetate for the polariscope.

"Although with undiluted urines containing large amounts of dextrose satisfactory results have been obtained with this method, the extreme care necessary in titrating under these conditions makes it advisable to dilute such urine from five to ten times. It is preferable to examine specimens when fresh, but should it become necessary to employ preservatives, toluol, salicylic acid, or carbolic acid may be added in small quantities without markedly interfering with the reaction. Chloroform, on the other hand, must be avoided, as even in minute traces its presence vitiates the test.

"In calculating the percentage of sugar by the above method it must be remembered that the titer of the copper solution is unchanged by the addition of the solution of potassium sulphocyanate, and that the mixture represents Fehling's solution diluted five times. Each c.c. of the reagent will, therefore, be reduced by 1 mg. of sugar.

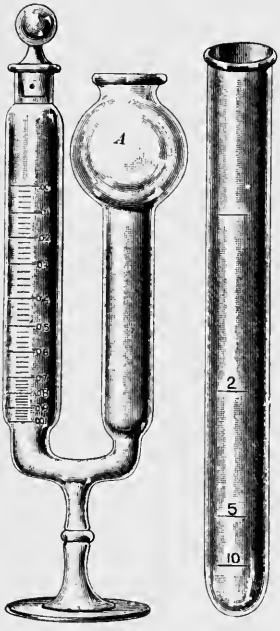


Fig. 160.—Lohnstein's saccharimeter.

"For example, if for the decolorization of 25 c.c. of the mixture, equivalent to 25 mg. of sugar, 1.2 c.c. of undiluted urine was used, then 1 c.c. of the urine will decolorize 25 divided by 1.2 = 20.8 c.c. of the reagent, equivalent to 28.8 mg. of sugar, or 2.08 per cent.

"If 0.75 c.c. of urine decolorize 25 c.c. of the reagent, 1 c.c. will decolorize 25 divided by 0.75 = 33.3 c.c. of reagent, equivalent to 33.3 mg. of sugar, or 3.33 per cent."

Einhorn's Method.—This will answer very well for ordinary purposes. Two especially constructed and graduated saccharimetric tubes (Fig. 159) are used, one of which is filled with a mixture of the suspected urine and yeast, and the other with normal urine and yeast, as a control. The examination in general is conducted as described before. (See Qualitative Tests for Sugar.)

Lohnstein's Method.—A very convenient modification of Einhorn's instrument, and one furnishing more accurate results, is that of Lohnstein. As will be seen from the accompanying figure (Fig. 160), this is essentially a U-tube open at both ends. The longer limb is closed during the process of fermentation by a ground-glass stopper. This stopper is provided with an air-hole, to which a similar hole corresponds in a drawn-out portion of the tube. The apparatus is filled with the urine to be examined, through the bulb *A*, while the two air-holes at *B* are in communication. Care should be had that the liquid stands exactly at the mark 0. The stopper is then turned so that all communication between the air and the urine is cut off. A little mercury is finally poured into the saccharimeter, when the instrument is maintained at a temperature of about 30° to 38° C. After twelve hours the percentage of sugar is read off directly.

Precautions.—1. As every urine contains traces of free carbon dioxide, it is well to remove this by boiling if we have reason to suppose that only a small amount of sugar is present. Before adding the yeast the urine is, of course, cooled to the surrounding temperature.

2. As the instrument yields satisfactory results only if the urine contains less than 1 per cent. of sugar, it is necessary to dilute it with water when more is present. The specific gravity may here serve as an index; urines of a specific gravity up to 1.018 are examined directly; from 1.018 to 1.022 they are diluted twice, from 1.022 to 1.028 five times, and those above 1.028 ten times.

3. A test-tube, provided with the necessary marks to indicate the degree of dilution of the urine, accompanies the instrument. In every case a globule of yeast, approximately 6 to 8 mm. in diameter, is added to the urine and shaken in the tube until an even suspension has been reached.[1] (See also Qualitative Tests for Sugar.)

Polarimetric Method.—For this purpose the saccharimeter of Soleil-Ventzke is very convenient (Fig. 161). This consists essentially of a Nicol prism at *A*, which may be rotated about the axis of the apparatus; a second Nicol prism, at *D*; vertically placed compensating prisms, consisting of dextrorotatory quartz, at *E*, which may be moved horizontally by means of a rack-and-pinion adjustment, turned by a milled head at *K*, so that light can pass through a thicker or thinner layer of the dextrorotatory quartz. At *F* is a plate of levorotatory quartz cut perpendicularly to the optical axis, and covering the entire field of vision; at *H* biquartz plates of Soleil, and at *I* an Iceland-spar crystal; *BC* represents a small telescope, by means of which the biquartz plates can be accurately focussed. When the compensation prisms of this apparatus are in a certain

[1] Lohnstein's saccharimeter may be procured from R. Kaltmeyer & Co., Oranjenburger Str. 45, Berlin.

position the levorotation of the plate *F* will be exactly compensated, and the two halves of the field of vision present the same color, while the zero of the scale *X* will coincide with the zero of the vernier *Y*, arranged on the upper surface of the compensators. Any change in this position produced by turning the screw *K* will cause the appearance of a different color in each half of the field of vision. If, now, with a zero position, an optically active dextrorotatory or levorotatory substance is interposed, the color of each half of the field of vision will become altered, but may be equalized again by changing the position of the compensators, the degree of change necessary to produce this result constituting an index of the power of rotation of the solution interposed in the tube *M*.

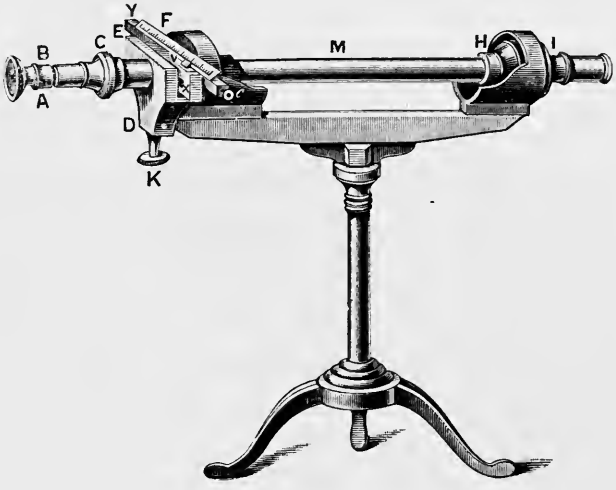


FIG. 161.—Soleil-Ventzke's saccharimeter.

Soleil-Ventzke's apparatus is constructed in such a manner that if a solution of glucose is employed, the length of the tube *M* being 10 cm., every entire line of division on the scale will indicate 1 per cent. of sugar.

The tube of the saccharimeter should be carefully washed out with distilled water, and at least once or twice with the filtered urine, when it is placed on end upon a flat surface and filled with the urine, so that this forms a convex cup at the end. The glass plate is now carefully adjusted, so as to guard against the admission of bubbles of air. The metallic cap is placed in position, care being taken to avoid undue pressure. The examinations are made in a dark room; an ordinary lamp is used, and several readings are taken, until the differences do not amount to more than 0.1 or 0.2 per cent. The tubes should be thoroughly cleansed *immediately* after the experiment.

In every case the filtered urine should be free from albumin, and, if markedly colored, should be previously treated with neutral lead acetate in substance and filtered.

If it is only desired to demonstrate the presence of sugar, the compensators are first brought to the zero position. If, now, upon interposition of the tube filled with urine a difference in the color of the two halves of the field of vision is noted, the presence of an optically active substance in the urine may be assumed; and if the deviation is at the same time to the right, the presence of glucose is rendered highly probable, while a deviation to the left will generally be referable to levulose or β–oxybutyric acid. Indican, peptones (albumoses), cholesterin, and certain alkaloids, it is true, also turn the plane of polarization to the left, but, as a rule, these substances need not be considered, as cholesterin occurs but rarely, and indican is usually present in only small amounts in diabetic urines. Albumoses, if present, must first be removed. Lactose and maltose, which also turn the plane of polarization to the right, may be distinguished from each other and from glucose by the phenylhydrazin test. Levulose turns the plane of polarization to the left. Oxybutyric acid is practically always associated with the presence of glucose, and may be recognized by allowing the urine to undergo fermentation, when the filtered urine will become distinctly levorotatory.

Lactose.—Lactose is a normal constituent of the urine during the last weeks of pregnancy and the first weeks following childbirth.

After lactation is once well established lactose is not usually found in the urine, but it may occur if for any reason milk stasis occurs.

Occasionally lactosuria is accompained by a mild grade of glucosuria.

A digestive lactosuria may follow the ingestion of 60 grams of lactose, though, as a general rule, 120 grams may be regarded as the limit of tolerance.

The presence of lactose may be inferred if a positive result is obtained with Trommer's and Nylander's tests, while the phenylhydrazin and fermentation tests give negative results. An osazone may, however, be obtained from the *isolated* substance.

Levulose.—An alimentary levulosuria occurs after the ingestion of more than 140 to 160 grams of sugar. In severe cases of diabetes levulose may be found in the urine together with glucose, even though the food contains neither levulose nor other carbohydrates. Such an occurrence is regarded as a grave omen.

Spontaneous levulosuria unaccompanied by glucosuria has also been described. Such urines show a deviation to the left or none at all, while the other tests for sugar indicate the presence of a reducing substance.

Maltose.—Maltose, together with glucose, was first found in the urine of a patient supposedly the subject of pancreatic disease, associated with an acholic condition of the stools. Since that time it has been repeatedly observed in diabetic patients. In one case the amount was 27.8 grams pro liter. Similar results have been obtained in dogs after extirpation of the pancreas. Its recognition is practically dependent upon the formation of its osazone and a determination of the melting point of the latter. Such urines, moreover, show a larger percentage of sugar on polarization than on titration with Fehling's solution. At the same time it will be observed that on heating for two hours with hydrochloric acid at 106° F., the polarimetric values become smaller, while the titration values increase.

Dextrin.—In one case of diabetes, dextrin appeared to take the place of glucose. It may be recognized by the fact that upon the application of Fehling's test the blue liquid first becomes green, then yellow, and sometimes dark brown. Traces of dextrin are probably present in every urine, but cannot be demonstrated with the common tests.

Laiose.—Laiose has been found in the urine of a diabetic patient. It is essentially characterized by the fact that on titration with Fehling's solution from 1.2 to 1.8 per cent. more sugar is indicated than by the polarimetric method.

Pentoses.—Traces of pentoses probably occur in every urine, but are not demonstrable by the common tests. Somewhat larger amounts may be found after the ingestion of fruit rich in pentoses, such as huckleberries, plums, cherries, etc. (digestive pentosuria). The tolerance of pentoses normally is less than 30 to 50 grams. If such amounts are taken one-half usually reappears in the urine.

Marked pentosuria has been described in a morphine habitué by Salkowski and Jastrowitz, where it alternated with glucosuria. Similar cases have been reported by Real, Külz, and Vogel, while others have observed pentosuria in diabetes. Several cases have been described in apparently normal individuals, and of late a family tendency to pentosuria has been observed in some cases. In these idiopathic cases arabinose is found, while xylose and rhamnose are met with in the digestive type of the anomaly.

Pentose urines reduce Fehling's solution and Nylander's solution, and give rise to the formation of an osazone when treated with phenylhydrazin. The osazone can be distinguished from that obtained from glucose, maltose, or lactose, etc., by the melting point (159° to 160° C.). The fermentation test is negative. Xylose and rhamnose turn the plane of polarization to the right, while arabinose is optically inactive. The presence of pentoses can be definitely established with the orcin test.

Orcin Test (Bial's Modification of Tollens' Test).—The reagent consists of 1 gram of orcin and 25 drops of the liquor ferri chloridi

in 500 c.c. of a 30 per cent. solution of hydrochloric acid. A few c.c. of this are heated to boiling in a test-tube and treated with a few drops of urine. A green color develops in the presence of pentoses. The green pigment can be extracted with amyl alcohol, and on spectroscopic examination it gives rise to a well-defined band of absorption in the red portion of the spectrum near the yellow border.

Tollens' Phloroglucin Test.—This test in which phloroglucin is substituted for the orcin, and in which a deep-red color is obtained in the presence of a pentose, may also be used, but the reagent indicates the presence of glucuronates as well.

GLUCURONIC ACID

Glucuronic acid is derived from glucose, and constitutes an intermediary product of the normal metabolism of the body. In the urine it is found only in combination with certain fatty and aromatic alcohols, forming compounds which are related to the glucosides and are generally spoken of as the *conjugate glucuronates*. Such bodies have been observed in the urine following the ingestion of chloral, camphor, naphthol, oil of turpentine, menthol, phenol, morphine, antipyrin, etc., and traces may also be obtained from normal urines. The normal glucuronates are undoubtedly compounds of glucuronic acid with phenol, paracresol, indoxyl, and skatoxyl. Their amount is exceedingly small, as the greater portion of these bodies is normally eliminated in combination with sulphuric acid. According to P. Mayer, an increased elimination of glucuronates precedes digestive glucosuria. Both conditions frequently co-exist in diabetic individuals.

Of the quantitative variations of the normal glucuronates and their relation to disease, next to nothing is known. Their clinical interest centres in the fact that certain glucuronates are capable of reducing copper and bismuth in alkaline solution. The glucuronates are readily decomposed by boiling with 1 per cent. H_2SO_4 (for one to five minutes). Unless this is previously done reduction of the alkaline copper sulphate solution only takes place slowly on prolonged heating. But if the cleavage is first accomplished it occurs at once. Such urines do not undergo fermentation. The glucuronates turn the plane of polarization to the left, while glucuronic acid itself is dextrorotatory. Like the pentoses, the glucuronates give a positive reaction with phloroglucin, while they do not react with orcin (see above). With the free acid phenylhydrazin forms crystalline compounds.

A quantitative method has been devised by Neuberg and Neumann,[1] but is too complicated for clinical purposes.

[1] Zeit. f. physiol. Chem., 1905, xliv, 127.

INOSIT

According to Hoppe-Seyler, traces of inosit may be found in the urine under normal conditions. Somewhat larger quantities are eliminated following the ingestion of large amounts of water, and for this reason possibly inosituria is notably observed in cases of diabetes insipidus, in diabetes mellitus, and in chronic interstitial nephritis. Its occurrence in these diseases is, however, not constant. The substance is devoid of clinical interest. It is not a carbohydrate, but belongs to the aromatic series, and is commonly regarded as hexahydroxybenzol. Its formula is $C_6H_{12}O_6 + H_2O$. For methods of isolating the substance from the urine, the reader is referred to special works.

URINARY PIGMENTS AND CHROMOGENS

Under normal conditions urochrome and uroerythrin, to which latter the red color of urate sediments is due, are the only pigments which occur preformed in the urine. In disease, on the other hand, various other pigments may be found, which occur either free or in the form of chromogens. Among the former may be mentioned hemoglobin, methemoglobin, hematin, hematoporphyrin, urorubrohematin, urofuscohematin, urobilin, the biliary pigments, and melanin; while abnormal chromogens are met with following the ingestion of certain drugs, such as santonin, senna, rheum, iodine, etc., as also in cases of poisoning with carbolic acid, creosote, etc. The occurrence of some of these substances, such as the various forms of blood pigment, the biliary pigments, and indigo, viz., indican, is of considerable clinical interest, while others again are of only minor importance.

Normal Pigments.—Urochrome.—To the presence of this pigment, which appears to be identical with the *normal urobilin of MacMunn*, but which should not be confounded with the *pathological urobilin of Jaffé*, the normal yellow color of the urine is probably largely due. It is supposedly derived from bilirubin, which in turn is referable to hematin, and thus from the hemoglobin of the blood.

Uroerythrin.—Uroerythrin is the pigment which imparts the red color to crystals of uric acid and the pink tint to urate sediments. Under strictly normal conditions it probably does not occur in the urine, but it readily appears with the slightest deviation from health, and when present in larger amounts, imparts a deep-orange color to the urine. Under pathological conditions it is seen especially in cases of hepatic insufficiency, in which the liver, owing to a greatly, increased destruction of red corpuscles, is unable to transform into bile pigment all the blood pigment which is carried to it. It also occurs when an absolute insufficiency on the part of the hepatic cells

exists, so that the organ is not even capable of causing the transformation of a *normal* amount of hemoglobin. Uroerythrin is seen in notable quantities in cases of cirrhosis and carcinoma of the liver, in passive congestion resulting from heart disease, in acute articular rheumatism, gout, pneumonia, malarial fever, erysipelas, spinal curvature, etc. In typhoid fever a marked excretion of uroerythrin is exceptional, and its occurrence has been associated with pulmonary complications. In nephritis it is seldom found in the urine, but Garrod cites an instance of pneumonia in which an abundant excretion of the substance accompanied conspicuous albuminuria.

In certain diseases, such as hepatic cirrhosis, the excretion of uroerythrin, as also of urobilin, is said to be much diminished when the patient is placed upon a milk diet (Riva).

When present in large amounts uroerythrin is readily recognized by the salmon-red color which it imparts to urinary sediments. Otherwise it is best to precipitate the urine with neutral lead acetate, barium chloride, or a similar reagent, when in the absence of uroerythrin a milky-white precipitate is obtained, while a pale rose-colored sediment indicates the presence of the pigment in appreciable amounts; a more pronounced rose color is produced if large quantities are present. In every case at least ten to fifteen minutes should be allowed to elapse before forming a definite conclusion, so that the sediment may have abundant time to settle.

Normal Chromogens.—The chromogens occurring in normal urine are indican, urohematin, and an unknown chromogen which yields urorosein when treated with mineral acids.

Indican.—Indican is the potassium or sodium salt of indoxyl sulphate, and hence a derivative of indol.

Formerly it was thought that indican could be formed within the tissues of the body in the absence of putrefactive organisms. Further researches, however, have demonstrated that microörganisms are always concerned in the production of indican, and that in health the large intestine is its sole source. Baumann, who succeeded in disinfecting the intestinal tract of a dog by means of large doses of calomel, observed that all traces of indican, as also of phenol and paracresol, disappeared from the urine. According to Senator, moreover, indican does not occur in the urine of newly born infants which have not as yet received nourishment. Tuczek's observations on abstinence from food in cases of insanity, in which indican was observed in the urine only when albumins, though in minimal amounts, were ingested, also speak very strongly against the older theory. Finally, it has been demonstrated that in cases in which an artificial anus is established near the distal end of the ileum the conjugate sulphates disappear almost entirely from the urine, while they reappear in normal amount as soon as the connection between the small and large intestines has been reëstablished.

The amount of indican which is normally eliminated in the urine varies somewhat with the character of the diet. Jaffé obtained 6.6 mg. from 1000 c.c. of urine as an average of eight observations. The largest quantities excreted in health are found after a liberal indulgence in animal food, while the smallest amounts are observed during a milk or kefir diet. By means of the latter article, indeed, the greatest diminution in the degree of intestinal putrefaction may be effected in man.

In pathological conditions an increased elimination of indican is observed:

1. In all diseases which are associated with an increased degree of intestinal putrefaction. As there appears to be little doubt that this is largely regulated by the acidity of the gastric juice, an increased indicanuria is encountered when anachlorhydria or hypochlorhydria exists. Large quantities of indican are thus eliminated in cases of carcinoma of the stomach, and exceeded only by those observed in ileus. Exceptions to this rule are at times, though rarely, met with, for which it is impossible to account at present. Large quantities of indican are also observed in cases of acute, subacute, and chronic gastritis. In the course of personal observations in this direction I was impressed with the curious phenomenon that in cases of ulcer of the stomach, notwithstanding the simultaneous occurrence of hyperchlorhydria, an increased elimination of indican, contrary to what is usually seen in hyperchlorhydria referable to other causes, is quite commonly found.

2. It should be noted that in cases in which the peristaltic movements of the *small* intestine have become impeded, as in ileus, acute and chronic peritonitis, an increased elimination of indican will invariably take place, no matter what the state of the gastric juice may be. In such conditions, and especially in ileus, the largest quantities are observed, a point which may be of *decided* value in differential diagnosis, as diseases of the large intestine alone are *never* associated with an increase in the amount of indican. *In simple, uncomplicated constipation increased indicanuria is not seen;* and should an examination in such cases reveal the presence of more indican than normal, it will be safe to assume the existence of disease elsewhere, and especially of the stomach.

3. As albuminous putrefaction may also take place within the body, an increased indicanuria may be observed in cases of empyema, putrid bronchitis, gangrene of the lung, etc.; but while in the conditions mentioned above the indol-producing organisms appear to be especially active, the elimination of phenol in the latter condition may be more pronounced at times than that of indican. Bearing in mind the points here set forth, I cannot agree with others in saying that the study of indicanuria possesses no importance from a clinical standpoint. I maintain, on the other hand, than *an examination*

of the urine in this direction is at least as important as the testing for albumin and sugar, and that points of decided importance, not only in diagnosis, but also in treatment, may thus be gained.

Of interest in this connection is the observation that in cases of increased indicanuria oxalate sediments are not uncommonly observed; but I am not willing to admit, as Harnack and van der Leyen suggest, that the indicanuria which follows the ingestion of small doses of oxalic acid is produced by a toxic action of the acid upon the tissue albumins. In these cases also the increased indicanuria must be referable to increased intestinal putrefaction.

When indican is treated with hydrochloric acid, it is decomposed into sulphuric acid and indoxyl; should an oxidizing substance be present at the same time, indigo blue, the blue coloring matter of the urine, results:

$$\underset{\text{Potassium indoxyl sulphate.}}{2C_8H_6NKSO_4} + 20 = \underset{\text{Indigo blue.}}{C_{16}H_{10}N_2O_2} + 2HKSO_4.$$

Indigo blue in small amounts may be found free in the sediment of decomposing urines, usually occurring in the form of small, amorphous granules, more rarely in crystalline form. Urines have, however, also been observed which were blue when passed, or which turned blue as a whole upon standing. Such a phenomenon must be regarded as a medical curiosity. Undoubtedly it is referable to the action of microörganisms (see Bacteriuria), although McPhedran and Goldie mention that in their case bacteria were present only in small numbers.

The blue pigment which may be obtained from urines has been variously described as Prussian blue, urocyanin, cyanurin, Harnblau, uroglaucin, choleraic urocyanin, but it has been shown to be indigo blue, and derived from its colorless antecedent indican. This has been shown to be identical with the uroxanthin of Heller and Thudichum's choleraic urocyanogen.

Tests for Indican.—A few cubic centimeters of urine are mixed with an equal volume of Obermayer's reagent, and shaken with a small amount of chloroform, which takes up the indigo blue that is formed. The resultant extract is normally either colorless or of a light sky blue; a darker color indicates an increased amount of indican. *Obermayer's reagent* is a 2 pro mille solution of ferric chloride in concentrated hydrochloric acid.

Stokvis' modification of Jaffe's test may also be employed. To this end a few cubic centimeters of urine are treated with an equal volume of concentrated hydrochloric acid, and 2 or 3 drops of a strong solution of sodium or calcium hypochlorite. The mixture is shaken with 1 or 2 c.c. of chloroform as above. The indigo which is set free in this manner is taken up by the chloroform, and colors this blue to a greater or less extent, the degree of increase, as com-

pared with the normal, being determined by the intensity of the color. Albumin need not be removed. Bile pigment, which interferes with the reaction, is removed by means of a solution of lead subacetate. Urines presenting a very dark color may be cleared in the same manner. Potassium iodide, owing to the liberation of free iodine, will color the chloroform a rose red.

For the sake of comparison, it is well to employ the same quantities of urine and reagents in every case, marked tubes being very convenient for this purpose.

For a consideration of methods for estimating the amount of indican the reader is referred to special works on physiological chemistry

Urohematin.—Urohematin appears to be the chromogen of the red pigment of the urine, and is very likely closely related to indoxyl. Little is known of its chemical composition or of its mode of formation. In all probability the red pigment which may be obtained from this substance is identical with other red pigments which have been described from time to time as occurring in the urine, such as that of Scherer, the urrhodin of Heller, the urorubin of Plosz, Schunk's indirubin, Bayer's indigo purpurin, Giacosa's pigment, and also the indigo red obtained by Rosenbach and Rosin by oxidation of the urine with nitric acid.

Further investigations are necessary before this subject is fully understood; but bearing in mind the probable origin of urohematin from indoxyl, it would possibly be best to speak of the red pigment as indigo red. In accordance with the view that urohematin is an indoxyl derivative, its clinical significance is similar to that of indican (which see).

Test.—The presence in normal urine of urohematin—*i. e.*, a chromogen yielding a red pigment when treated with certain reagents—may be demonstrated by shaking urine with chloroform and decanting after several days, when the addition of a drop of hydrochloric acid to the chloroform extract will cause the appearance of a beautiful rose color; this varies in intensity according to the amount of the chromogen present.

The purplish color so often obtained in the chloroform extract when Stokvis' modification of Jaffé's indican test is employed is due to a mixture of indigo blue and indigo red. Indican, however, is generally present in larger amounts than urohematin. In normal and, usually also, in pathological urines a red color is not obtained with the test mentioned. In a few isolated cases of ileus, peritonitis, and carcinoma of the stomach I have found more indigo red than indigo blue.

The so-called "Reaction of Rosenbach" is a convenient test for indigo red when this is present in increased amounts; the boiling urine is treated drop by drop with concentrated nitric acid, when in the presence of large amounts of indigo red it assumes a dark

Burgundy color, which sometimes takes on a bluish tinge when held to the light. Owing to a precipitation of the pigment the mixture at the same time becomes cloudy and the foam assumes a blue color. In well-marked cases the Burgundy color does not appear to be changed by the further addition of nitric acid, but will sometimes suddenly change from red to yellow when 10 to 20 drops of the acid have been added.

This reaction Rosenbach regarded as symptomatic of various forms of severe intestinal disease associated with an impeded resorption throughout the entire intestinal tract. Ewald likewise noted this reaction in cases of extensive disease of the small intestine, in carcinoma of the stomach, and in acute and chronic peritonitis; but he obtained negative results in carcinoma of the colon, stricture of the esophagus, chronic diarrhea, etc. *Rosenbach's reaction should be viewed in the same light as a highly increased elimination of indican.* I have met with the reaction in all conditions associated with greatly increased intestinal putrefaction, and, like Ewald, failed to note the reaction in a few cases of occlusion of the large intestine, in which an increased elimination of indican is likewise never observed.

Uroroseinogen.—In addition to indican and urohematin, still another chromogen, which yields a rose-red pigment when treated with mineral acids, appears to occur in normal urine, although in small amounts. It is commonly regarded as a skatol derivative. The pigment, which has received the name *urorosein*, or Harnrosa, appears to be identical with Heller's urophain. Urorosein is best demonstrated by treating 5 to 10 c.c. of urine with an equal amount of concentrated hydrochloric acid, and 1 or 2 drops of a concentrated solution of sodium hypochlorite, when in the presence of much indican the mixture assumes a dark greenish, blackish, or dark-blue color, owing to the formation of indigo. When the mixture is shaken with chloroform the supernatant fluid exhibits a beautiful rose color, which is due to the urorosein. This may now be extracted with amyl alcohol and separated from other pigments which are present at the same time, by shaking with sodium hydrate, whereby the solution is decolorized. Upon the addition of a drop or two of hydrochloric acid to the alcoholic extract the rose color reappears. Such solutions, however, soon become decolorized upon standing. A rose-red ring referable to this pigment is also frequently obtained in pathological urines when the ordinary nitric acid test is employed.

While normally urorosein is obtained only in traces, appreciable amounts are often met with in pathological conditions associated with grave disturbances of nutrition, as in nephritis, diabetes, carcinoma, dilatation of the stomach, pernicious anemia, typhoid fever, phthisis, and at times in profound chlorosis, etc. A vegetable diet also appears to cause an increase in the amount of the chromogen.

Pathological Pigments and Chromogens.—The Blood Pigments.—The blood pigments proper which may occur in the urine have already been considered, and in this connection it will only be necessary to refer briefly to the occasional presence of hematin, urorubrohematin, and hematoporphyrin.

Hematin.—Hematin is only rarely found. In order to demonstrate its presence, the urine is rendered strongly alkaline with ammonia, filtered, and the filtrate examined spectroscopically. (See Blood.)

Urorubrohematin and Urofuscohematin.—These have been observed only once by Baumstark in the urine of a case of pemphigus leprosus complicated with visceral lepra; they appear to be closely related to hematin.

Hematoporphyrin.—McMunn found a pigment answering the description of this substance in the urine in cases of rheumatism, Addison's disease, pericarditis, and paroxysmal hemoglobinuria, which he termed urohematin, but which in all probability was hematoporphyrin. Le Nobel found the same pigment in two cases of hepatic cirrhosis and in one case of croupous pneumonia. Others have likewise met with hematoporphyrinuria in various forms of hepatic disease, as also in phthisis, exophthalmic goitre, typhoid fever, and hydroa æstivalis; further, in association with intestinal hemorrhages, in cases of lead poisoning, and especially during long-continued use of sulphonal, trional, and tetronal. Nebelthau records the history of a female patient, the subject of congenital syphilis, who had passed dark red urine as long as she could remember, and continued to do so while under observation. Stern mentions a case in which marked hematoporphyrinuria was associated with icterus in a glucosuric individual. Recent researches, moreover, have shown that in traces at least the substance is present in every urine. As regards the origin of these normal traces, the evidence is in favor of the view that they are formed within the body during its normal metabolism, and most likely in the liver, whence the substance is eliminated in the bile. A portion then escapes with the feces, while a similarly small amount is resorbed and eliminated in the urine. Increased amounts would accordingly suggest the existence of an hepatic insufficiency; and, as a matter of fact, we find that actual anatomical lesions then not infrequently occur. Taylor and Sailer thus report that in their case of sulphonal poisoning widespread degeneration of the hepatic cells existed; and Neubauer was able to isolate the pigment from the liver of rabbits to which sulphonal had been administered, while it was absent in all other organs. On the other hand, it is difficult to ascribe all the phenomena of such hematoporphyrinuria to hepatic changes, seeing that changes of like degree may occur without conspicuous urinary abnormality, and there is still much that is obscure in this condition.

Stokvis attributed the increased elimination of hematoporphyrin

in cases of lead poisoning and following the continued use of sulphonal to the occurrence of hemorrhages into the intestinal mucosa, and suggested that the transformation of the hemoglobin into hematoporphyrin was favored by the sulphonal. But while intestinal hemorrhages may occur in the sulphonal cases, they are not always observed, and, as Garrod points out, Kast and Weiss, as also Neubauer, were unable to verify the recorded experiments of Stokvis, in which he claims to have obtained a small amount of hematoporphyrin when fresh blood was digested with pepsin-hydrochloric acid and sulphonal at from 38° to 40° C.

Urines which contain much hematoporphyrin are usually dark red in color, but the shade may vary from a sherry or port-wine tint to a dark Bordeaux. It is noteworthy, however, that this color is not primarily due to the exaggerated degree of hematoporphyrinuria, but, as Hammarsten first pointed out, to other abnormal pigments which are but little known, but which are probably closely related to hematoporphyrin. As Garrod says, the removal of the hematoporphyrin from such urines causes little or no change of color, and when this pigment is added to normal urine until on spectroscopic examination bands of similar intensity are seen, the change of tint produced is comparatively slight. In one such case, not due to sulphonal, he was able to isolate a purple pigment which differed in its properties from any known urinary coloring matter, and to which the color of the urine in question was obviously in the main due. Neumeister also states that in sulphonal intoxication an iron-containing derivative of hemoglobin occurs in the urine, which presents a reddish-violet color and shows a single band of absorption in the blue portion of the spectrum immediately bordering on the green.

Albumin is not present in uncomplicated cases of hematoporphyrinuria, and the pigment itself does not give the albumin reactions.

Garrod's Method for Demonstrating Hematoporphyrin.—To demonstrate the presence of hematoporphyrin under normal conditions, or when small amounts only are present in the urine, Garrod's method should be employed.

Several hundred c.c. of urine (500 to 1500) are treated with a 10 per cent. solution of sodium hydrate in the proportion of 20 c.c. of the alkali solution for 100 c.c. of urine. The precipitated phosphates are filtered off and thoroughly washed by repeatedly suspending them in water. Should the precipitate be of a reddish color, or if it shows the spectrum of hematoporphyrin in alkaline solution when examined on the filter in the moist state, we may conclude that much hematoporphyrin is present. In this case it is washed until the filtrate is colorless. If traces only are present, however, one washing must suffice. The precipitate is then treated with alcohol, which is acidified with hydrochloric acid to such an extent that the phosphates are entirely dissolved. The resulting

solution should not exceed 15 to 20 c.c. in volume. This is then examined in a layer, of not less than 3 to 4 cm. in thickness, for the spectrum of acid hematoporphyrin, using a spectroscope with slight dispersion. The solution is now rendered alkaline with ammonia and treated with an amount of acetic acid which just suffices to redissolve the precipitated phosphates. On shaking with chloroform this extracts the pigment, and the chloroform solution then gives the spectrum of the alkaline hematoporphyrin, since organic acids do not change the pigment to the form which yields the acid spectrum. The residue which remains after evaporating the chloroform can finally be washed with water and dissolved in alcohol, when a nearly pure solution is obtained, which is comparable with a solution of hematoporphyrin obtained from hematin.

Precautions.—If a preliminary test shows that the urine contains but little phosphates, a small quantity of calcium phosphate in acetic acid is added before the urine is rendered alkaline with the sodium hydrate solution. As hematin and chrysophanic acid are also precipitated with the phosphates, their absence must be insured. For this reason the urine should contain no rhubarb or senna.

In conclusion, it may be said that a chromogen of hematoporphyrin is also usually present in urines containing the free pigment, which probably explains why such urines gradually become darker on standing.

Biliary Pigments.—Of the four biliary pigments, viz., bilirubin, biliverdin, biliprasin, and bilifuscin, the former alone is met with in freshly voided urines, while the others may form upon standing, being oxidation products of bilirubin. The pigment is never found in normal urine, and its occurrence may be regarded as a positive symptom of disease.

In health it will be remembered that *bilirubin* is formed in the liver from blood pigment, and is eliminated into the small intestine, in which it is transformed into hydrobilirubin and largely excreted as such in the feces, while a small portion is reabsorbed into the blood and eliminated in the urine as urochrome or normal urobilin. Whenever, then, the outflow of bile into the intestines becomes impeded bilirubin is absorbed by the lymphatics and eliminated in the urine.

Among the numerous causes which give rise to *choluria* under such conditions may be mentioned obstruction of the biliary ducts, and especially of the common duct, referable to simple swelling of its mucous membrane, as in the ordinary forms of catarrhal jaundice. It may also be due to the presence of a biliary calculus, to parasites, compression of the duct by tumors of the liver, the gall-bladder, the duct itself, and of neighboring structures, and particularly of the pancreas, stomach, and omentum. Whenever the blood pressure in the liver is lowered, so that the tension in the smaller biliary ducts becomes greater than that in the veins, choluria like-

wise results. The icterus occurring under all such conditions has been termed *hepatogenic icterus*, in contradistinction to the form observed in cases in which the liver has either totally or partially lost the power of forming bile, be this owing to the existence of degenerative processes affecting its glandular epithelium, as in cases of acute yellow atrophy, or to destruction of red corpuscles going on so rapidly and so extensively that the organ is incapable of transforming into bilirubin all the blood pigment which is carried to it. This occurs in some cases of pernicious anemia, malarial intoxication, typhoid fever, poisoning with arsenous hydride, etc. Icterus neonatorum is probably to a certain extent also dependent upon the latter cause. To this form the term *hematogenic icterus* has been applied. In such cases the occurrence of bilirubin in the urine can only be explained by assuming that a transformation of blood-coloring matter into bilirubin has taken place in the blood itself or in other tissues of the body. As a matter of fact, it appears to be generally accepted that such a transformation *can* occur outside of the liver, as the hematoidin which may be found in old extravasations of blood seems to be identical with bilirubin. On the other hand, however, the existence of a hematogenic icterus is positively denied, especially by Stadelmann. In accordance with his view it may be demonstrated that in cases of pernicious anemia, malaria, etc., the urine does not contain bilirubin, but usually urobilin. In cases of this kind which I had occasion to examine, bilirubin was, as a matter of fact, never found. Further investigations are necessary to settle this question.

Usually the presence of biliary pigment may be recognized by direct inspection, as urines which contain it in notable amounts present a color varying from a bright yellow to a greenish brown. Any morphological elements which may occur in the sediment are stained a golden yellow, and the same color is imparted to the foam of the urine as well as to the filter paper used in the filtration. At times, however, and particularly in cases in which the icterus is only beginning to appear, the presence of bilirubin is not infrequently overlooked, and urines containing urobilin in large amounts may be similarly mistaken for icteric urines. In doubtful cases, therefore, whether icterus exists or not, but in which the urine presents an intense yellow color, it is necessary to have recourse to chemical tests. A large number of these have been devised, all of which are fairly reliable. Only those will be described which I have examined myself and which are especially delicate.

Smith's Test.—5 to 10 c.c. of urine are placed in a test-tube and treated with 2 or 3 c.c. of tincture of iodin (which has been diluted with alcohol in the proportion of 1 to 10) in such a manner that the iodin solution forms a layer above the urine. In the presence of bilirubin a distinct emerald-green ring is seen at the zone of con-

tact. This test can be highly recommended, as it is exceedingly simple and not surpassed in delicacy by any other.

Huppert's Test.—10 to 20 c.c. of urine are precipitated with milk of lime, or a solution of barium chloride, and the precipitate after filtering brought into a beaker by perforating the filter and washing its contents into the latter with a small amount of alcohol acidulated with sulphuric acid. The mixture is boiled, when in the presence of bilirubin the solution assumes a bright emerald-green color. Huppert's test is as delicate as is that of Smith, but is not so convenient for the needs of the practising physician.

Gmelin's Test (as modified by Rosenbach).—The urine is filtered through thick Swedish filter paper, when the latter is removed and a drop of concentrated nitric acid, which has been allowed to stand exposed to the air for a short time, is placed upon its inner surface. In the presence of bilirubin a prismatic play of colors will be seen to occur around the nitric acid spot.

Gmelin's Test.—The urine is treated with nitric acid, which is carried to the bottom of the test-tube by means of a pipette, so as to form a layer beneath the urine, when a color play will take place at the line of contact between the two fluids; the green color is the most characteristic.

In this connection a few words may also be said of the occurrence in the urine of biliary acids and cholesterin.

Biliary Acids.—These may usually be found in the urine whenever bile pigment is present, so that their clinical significance is essentially the same as that attaching to bilirubin. Their demonstration is, however, attended with much difficulty. (See Feces.)

Cholesterin.—Cholesterin has never been found in icteric urines, and is only rarely seen in other pathological conditions. It has been observed in cases of chyluria, fatty degeneration of the kidneys, diabetes, in one case of epilepsy, in eclampsia, and in several cases of pregnancy. v. Jaksch noted cholesterin crystals in a urinary sediment in a case of tabes with cystitis. Glinsky records a similar observation. Harley found it repeatedly in cases of pyuria. Reich states that he found cholesterin crystals of the size of a dollar in the urine of a case of chronic cystitis. Hirschlaff found larger quantities in the urine of a case of hydronephrosis; on one occasion 5.8 grams in 100 c.c. of urine. I have found cholesterin crystals in the sediment in a case of acute nephritis. Güterbock described a urinary calculus obtained from the bladder of a woman which consisted almost entirely of cholesterin. (See Feces.) Langgaard noted the presence of the substance in a case of chyluria.

Pathological Urobilin.—This pigment should not be confounded with the urochrome or normal urobilin described above, to which it is closely related, but from which it may be distinguished by means of the spectroscope. Gautier states that pathological urobilin may

be obtained from urochrome by submitting the latter to the action of reducing agents: and, as I have already pointed out, Riva and Chiodera obtained a substance from urobilin by the action of potassium permanganate, which closely resembles urochrome. It is said to be identical with the *stercobilin* found in the feces, but differs from Maly's hydrobilirubin in containing a much smaller percentage of nitrogen, viz., 4.11, as compared with 9.22 (Garrod and Hopkins). While its occurrence in the urine is essentially a pathological phenomenon, it is at times also met with in normal urine, and appears to be derived from a special chromogen, *urobilinogen*, from which it may be set free by the addition of an acid. Both urobilin and its chromogen are precipitated by saturating the urine with ammonium sulphate, and both are soluble in chloroform. According to Maly, urobilin is formed by the reduction of bilirubin in the intestine, and is then in part resorbed and eliminated in the urine. Hayem, on the other hand, proposed the hypothesis that the substance originates in a diseased or disordered liver, as bilirubin does in the same organ in health, and accordingly he regards the appearance of much urobilin in the urine as evidence of hepatic insufficiency. Others, again, maintain that urobilin is formed in the tissues at large either by the reduction of bilirubin or directly from the blood pigment. The first view is notably held by Kunkel, Mya, Giarré and others, while the hematogenous theory was notably represented by Gerhardt. Garrod discusses these various hypotheses at some length in his most interesting lecture on the urinary pigments in their pathological aspects, in which he personally inclines to the intestinal theory, as now held by Müller, Schmidt, Esser, and others. In a work of this scope it would lead too far to discuss the various investigations which lend themselves in support of this view, and I can here quote only the following from Garrod's paper: "The chief seat of the formation of urobilin (for it is convenient to employ this term as including both pigment and chromogen) is undoubtedly the intestinal canal. This can only be gainsaid by denying the identity of the urinary and fecal pigments. The quantity normally present in the feces is far larger than that which enters the intestine with the bile (when a small amount is found), and there is strong evidence that the urobilin in bile is itself of intestinal origin. This being so, it is clear that theories other than the intestinal and its modifications merely attempt to trace a second source for the urobilin of the urine. It is equally clear that the substance from which the intestinal urobilin is formed is the bile pigment. Under ordinary conditions the bile pigment is destroyed in its passage along the intestine, and does not appear as such in the feces. In its place we find large quantities of urobilin, which in its turn disappears when occlusion of the common duct prevents the entrance of bile into the intestine. Again, when under certain morbid conditions the bile pigment passes along the intestine

unaltered, urobilin is absent from the feces. However, the conversion of bilirubin into urobilin is no mere process of reduction, but involves a much more radical change, with elimination of nitrogen. That the change is brought about by bacterial action there is much evidence to show. When bile is inoculated with fecal material and kept in an incubator a formation of urobilin rapidly takes place, and at the same time the bile pigment diminishes, and ultimately disappears."

From its frequent occurrence in febrile urines pathological urobilin has also received the name *febrile* urobilin.

Its presence is very common in hepatic cirrhosis. In 12 cases of the atrophic and hypertrophic variety v. Jaksch was able to demonstrate urobilin in every instance, a point which may at times be of considerable diagnostic importance. I have observed urobilin in a few cases of hepatic cirrhosis, chronic malaria, and pernicious anemia, in all of which the skin presented a light icteric hue, and in which bile pigment was absent from the urine. Unfortunately, an examination of the blood was not made, and I have hence not been able to confirm the statement of v. Jaksch that bilirubin occurs in the blood in almost every case in which urobilin is present in the urine. Syllaba, however, has shown that in pernicious anemia urobilinuria is quite constantly associated with bilirubinemia (see the latter). Urobilin has also been noted in cases of carcinoma, scurvy, Addison's disease, hemophilia, in cases of retro-uterine hematocele, in extra-uterine pregnancy, following intracranial hemorrhages, etc. According to Bargellini, the degree of constipation in simple atony of the bowel is without influence upon the amount of urinary urobilin, but he states that in typhoid fever it causes an obvious increase; whereas disinfection or emptying of the large bowel produces a notable diminution in the amount. Urobilinuria, according to Samberger, is common early in secondary syphilis and referable to increased destruction of red cells. In some cases the urobilinuria, is only observed after the mercurial treatment has been instituted, and subsequently disappears.

Urines rich in urobilin usually present a dark-yellow color which is strongly suggestive of the presence of bilirubin; even the foam in such cases may be colored, making the resemblance between the two pigments still more complete. This dark color, however, is not due to urobilin, but to associated pigments.

Gerhardt's Test.—If the urine contains much urobilin, which the color will indicate, 10 to 20 c.c. are extracted with chloroform by shaking, and the extract treated with a few drops of a dilute solution of iodopotassic iodide. Upon the further addition of a dilute solution of sodium hydrate the chloroform extract is colored a yellow or yellowish brown, and exhibits a beautiful green fluorescence; this is even more intense than that noted in the case of normal urobilin.

Braunstein's Test.—The reagent is composed of 100 c.c. of a concentrated solution of copper sulphate, 6 c.c. of concentrated hydrochloric acid, and 3 grams of ferric chloride; 20 c.c. of urine are treated with 3 or 4 c.c. of the reagent and shaken with chloroform. In the presence of urobilin a rose to a red color develops.

Schlesinger's Test.—10 c.c. of urine are treated with an equal quantity of a 1 per cent. solution of acetate of zinc in absolute alcohol. The mixture is agitated and filtered, when in the presence of urobilin the filtrate will show distinct fluorescence.

Spectroscopic Examination.—The urine is best examined as follows: 50 c.c. of urine are extracted in a separating funnel with amyl alcohol, which takes up both the pigment and its chromogen. After standing for several hours the urine is allowed to flow away by opening the stopcock, when the alcoholic extract is decanted from above, and is treated with a concentrated alcoholic and ammoniacal solution of zinc chloride. In the presence of urobilin the liquid shows a beautiful fluorescence, and on spectroscopic examination a single band of absorption is seen between *b* and *F*. In acid solutions, on the other hand, a single band is likewise obtained between *b* and *F*, but this extends to the right beyond *F*, and is much darker. Should the urine contain much urobilin, its special extraction is not necessary. In such an event the acid urine shows the acid spectrum, while the alkaline band is obtained after the addition of ammonia. (See also Bang's Test.)

Melanin and Melanogen.—In cases of melanotic disease it has been repeatedly observed that the urine, which usually and probably always presents a normal yellow color when voided, gradually becomes darker upon exposure to the air, and finally turns black. Such urines generally contain melanin and its chromogen in solution; deposits of melanin granules by themselves are only occasionally seen, and are not characteristic, as they may also be found in cases of chronic malarial intoxication, etc.

While the occurrence of melanin in the urine is probably indicative in most cases of the existence of melanotic tumors, it should be stated that this symptom cannot be regarded as pathognomonic, as it may be absent in the case of melanotic tumors, and present in wasting diseases and inflammatory affections, and may at times, though very rarely, be associated with non-pigmented growths. Nevertheless, its occurrence should always be regarded with suspicion, and, taken in conjunction with other symptoms will often lead to a correct diagnosis.

Tests for Melanin and Melanogen.—1. The presence of melanogen may be assumed if upon the addition of ferric chloride solution a black precipitate appears in the urine, which is soluble in a solution of sodium carbonate, and can be reprecipitated as a black or brownish-black powder by mineral acids. Instead of ferric chloride barium hydrate may also be used.

2. A few cubic centimeters of urine are treated with bromine water, when in the presence of melanin or melanogen a precipitate is obtained, which is yellow at first, but gradually turns black.

Phenol.—Phenol, according to Brieger, occurs only in very small amounts in human urine, the usual phenol reactions being largely referable to paracresol. Normally, about 0.03 gram is eliminated in the twenty-four hours, but in pathological conditions much larger quantities may be found. Remembering the origin of phenol, it is clear that an increased elimination may be observed whenever putrefactive processes are going on in the tissues and cavities of the body, or whenever there is an increase in the degree of intestinal putrefaction, though in the latter condition the indican is usually the only conjugate sulphate that is found increased. In peritonitis, diphtheria, erysipelas, scarlatina, empyema, pulmonary gangrene, putrid bronchitis, etc., an increased elimination of phenol is commonly seen, as also in certain cases of pernicious vomiting of pregnancy. Important from a diagnostic standpoint, further, is the fact that in uncomplicated cases of typhoid fever no increase is observed, while this is common in tuberculous meningitis. The largest amounts, of course, are seen in cases of poisoning with carbolic acid or one of its derivatives (hydroquinone, pyrocatechin, salicylic acid), where the urine may darken on standing, thus simulating true melanuria.

As the quantitative estimation of phenol is too complicated for the purposes of the general practitioner, Salkowski's qualitative test only is here described. From the intensity of the reaction certain conclusions may be drawn as to the amount present. It is especially serviceable in cases of suspected poisoning with carbolic acid.

Salkowski's Test.—About 10 c.c. of urine are boiled in a test-tube with a few cubic centimeters of nitric acid, and, on cooling, treated with bromine water. The development of a pronounced turbidity or the occurrence of a precipitate indicates the presence of an increased amount of phenol.

Salol and Salicylic Acid.—These may be recognized from the fact that such urines when treated with a solution of ferric chloride develop a marked violet color which does not disappear on standing. The reaction thus differs from that obtained with diacetic acid.

Alkapton.—Urines are at times, though very rarely, seen which, like the phenol urines, turn dark on standing, but in which the change in color is neither referable to the presence of phenol or its derivatives, nor to melanin. Such urines are of a normal color when passed, but gradually turn reddish brown upon exposure to the air. Treated with a small amount of alkali, this change occurs almost immediately. Fehling's solution is reduced on the application of heat, while bismuth is not affected. Ammoniacal silver solution is reduced in the cold, and a temporary bluish-green color develops when the urine is treated with a ferric salt. The fermenta-

tion test is negative, and examination with the polarimeter shows that the substance in question is not glucose. With phenylhydrazin no osazone is formed.

Bödeker, who first observed a urine of this kind, termed the substance giving rise to the reaction just described alkapton, and subsequently expressed the belief that his alkapton might possibly have been pyrocatechin. Subsequent investigators succeeded in isolating substances from such urines which have been variously termed pyrocatechuic acid, urrhodinic acid, glucosuric acid, uroleucinic acid, and uroxanthinic acid. Baumann and Wolkow later were able to isolate *homogentisinic acid* in pure form from the urine of such cases, and expressed the belief that some of the substances obtained by previous observers were in reality the same. Since that time this acid has also been found by Garrod, Ogden, Stange, Stier and others.

Of the origin of alkapton little is known. Baumann expressed the opinion that homogentisinic acid might be derived from tyrosin, and that the condition is referable to the activity of special microorganisms in the upper portions of the intestines. As a matter of fact, the amount of homogentisinic acid can be materially increased by the administration of tyrosin, and Mittelbach has shown that if the substance is given in frequently repeated and small doses, almost the entire amount reappears in the urine as homogentisinic acid. Tyrosin, however, belongs to the *para-* series, while homogentisinic acid is an *ortho-* compound, so that the transformation of tyrosin into homogentisinic acid cannot be a direct process, and it has accordingly been questioned whether Baumann's view regarding the origin of alkapton is correct. There is evidence indeed to show that homogentisinic acid does not originate in the intestines, viz., is not a product of bacterial activity. It has thus been found that the alkaptonuria does not cease during starvation, and that a restriction of the putrefactive processes in the intestines by means of oil of turpentine, a kefir diet, and the administration of β-naphthol does not lead to a diminished elimination of homogentisinic acid. It has never been found in the feces, moreover, and Garrod has shown that after inoculation of common bouillon, meat juice, or tyrosin broth with alkaptonuric feces homogentisinic acid is not formed. On the other hand, Embden observed that when an alkaptonuric individual took homogentisinic acid by the mouth a far larger portion appeared in the urine than when the same substance was administered to a healthy individual, which suggests that the alkaptonuria may be referable to impairment of the normal processes of oxidation. Very significant is the discovery that a notable increase follows the administration of phenylalanin, and that the ingestion of phenylacetic acid will increase the power of reduction and of rotation of the urine. Phenylpropionic acid and benzoic acid cause no increase in the elimination of homogentisinic acid.

The prevailing view is that alkaptonuria is a metabolic anomaly comparable to glucosuria and cystinuria; but, unlike glucosuria, it can scarcely be regarded as an expression of a pathological process. It may, of course, occur in individuals, suffering from disease, and has been observed in connection with glucosuria, in acute gastro-intestinal catarrh, in phthisis, acute miliary tuberculosis, in one case of brain tumor, carcinoma of the prostate, etc. More frequently the condition is accidentally discovered in apparently healthy individuals, and has repeatedly been confounded with glucosuria owing to the positive reduction test with Fehling's solution.

Garrod, from an analysis of all the reported cases, concludes that the condition is nearly always congenital. In 32 known instances which were presumably congenital, 19 occurred in seven families. One family contained 4 alkaptonurics, three others 3, and the remaining three 2 each. In fully 60 per cent. of the cases, it appears from Garrod's studies, the parents of alkaptonurics were first cousins. There is thus far only one known instance in which the anomaly has been transmitted by an alkaptonuric father to his son.

The condition commonly persists through years and perhaps a lifetime. It may also occur as a transitory abnormality, however, as is apparent from the case of Hirsch, in which the condition persisted for three days, and the case of Geyger, in which the alkaptonuria was observed on only two days. A few observers further report the occurrence of alkaptonuria shortly preceding death.

Very interesting in this connection is the observation of Osler and others that the urine of patients with ochronosis will darken on standing and may contain homogentisinic acid. The pigmentation of the cartilages thus seemed to be a possible morphological expression of the urinary abnormality. But as Garrod has already stated, it is possible also that other substances besides homogentisinic acid may cause the blackening of the urine in ochronosis.

The amount of homogentisinic acid eliminated in the twenty-four hours is variable, but usually large. Baumann found an average elimination of 4.6 grams; the largest amount in twenty-four hours was 6 grams. In Meyer's case, a child one and one-half years old, 3.3 grams were passed pro die. Larger quantities are obtained after a liberal diet of meats than with a vegetable diet.

Isolation and Estimation (*Garrod's Method*).—The urine is heated nearly to boiling without any preliminary treatment, and for each 100 c.c. at least 5 or 6 grams of solid neutral lead acetate are added.

As soon as the acetate is dissolved, the bulky gray precipitate which forms is removed by filtration, and the filtrate, which has a pale-yellow color, is allowed to stand for twenty-four hours in a cool place. If the urine be very rich in homogentisinic acid, or if the flask, containing the filtrate be placed upon ice, minute acicular crystals, which are almost colorless, quickly form; but, as a rule,

crystallization does not commence until several hours have elapsed. The crystals are then much larger, are grouped in stars or rosettes, and are more deeply colored.

In summer weather it would probably be desirable to start the crystallization by artificial cooling; but although the process is greatly accelerated at a low temperature, the total yield is not materially increased.

If the formation of the crystals be long delayed, the liquid may be warmed again and more lead acetate added.

After the lapse of twenty-four hours crystals cease to form, even when the liquid is placed upon ice.

The crystalline product so obtained is lead homogentisinate. When the crystals are dissolved in hot water the solution assumes a deep brown color with alkalies; it reduces Fehling's solution readily with the aid of heat, and yields a transitory deep blue color with a dilute solution of ferric chloride. From the lead salt free homogentisinic acid may be obtained by decomposing it with hydrogen sulphide.

For clinical purposes the following method also may be employed:

Baumann's Method.—50 c.c. of urine are treated with 15 grams of ammonium chloride, which should be brought into solution by shaking, in a stoppered graduate. After standing for about twelve hours to allow the uric acid to separate out the solution is filtered and an accurately measured portion of the filtrate titrated with a decinormal ammoniacal solution of silver nitrate. The titration is continued until a further reduction of the silver solution does not occur, which is ascertained by acidifying a few drops of the filtered mixture with hydrochloric acid, when in the presence of free silver a turbidity referable to silver chloride occurs. Accuracy within narrower limits than $\frac{1}{4}$ c.c. is scarcely possible, as the turbidity referable to silver chloride can only be recognized within 0.2 to 0.3 c.c. According to Baumann, 240 to 245 c.c. of the silver solution represent 1 gram of homogentisinic acid.

Blue Urines.—Blue urines are sometimes seen, the color of which is due to indigo formed from urinary indican within the urinary passages. Their occurrence can only be regarded as a medical curiosity. One case of this kind is reported by McPhedran and Goldie, in which after direct extraction of the urine with ether only a faint reaction was obtained on further examination, and which probably was referable to incomplete previous extraction. Formerly, when indigo was employed in the treatment of epilepsy, blue urines were frequently seen. At the present time, when methylene blue is occasionally used in the treatment of malaria and chyluria, this pigment is found in the urine.

Green Urines.—Green urines have also been described; the cause of the color, however, has not been ascertained.

Pigments Referable to Drugs.—Certain drugs may also cause changes in the normal color of urine, and in doubtful cases inquiry in this direction should be made. It has been pointed out that carbolic acid, hydroquinone, pyrocatechin, and salol cause the appearance of a dark brown color, and that after the administration of indigo and methylene blue, blue urines are voided. Santonin, rheum, and senna color urines a bright yellow, so that they may resemble icteric urines. The yellow color in such cases is changed to an intense red by the addition of an alkali, and, if ammoniacal fermentation is going on at the same time in the bladder, the patient may believe himself to be suffering from hematuria. The red color thus produced is due to the action of the alkali upon chrysophanic acid. When urines containing copaiba are treated with hydrochloric acid a red color results, which changes to violet upon the application of heat. During the administration of potassium iodide, or the use of iodine in any form, a dark mahogany color is obtained when the urine is treated with nitric acid. In doubtful cases Stokvis' modification of Jaffé's test for indican should be employed, when in the presence of an iodide the chloroform assumes a beautiful rose-red color.

For the detection of other drugs and poisons in the urine the reader is referred to special works.

Ehrlich's Diazo Reaction (Plate XXIV).—Under certain pathological conditions, especially in typhoid fever, a chromogen may be present in the urine, which when treated with diazo-benzene-sulphonic acid and ammonia imparts a red color to the urine, varying from eosin to a deep garnet red. This reaction, which is generally spoken of as Ehrlich's reaction, or the *diazo reaction*, was at one time regarded as pathognomonic of typhoid fever. Subsequent examinations, however, have shown that it may also be present in other diseases. Michaelis, who has made an exhaustive study of this question, divides into four groups the diseases in which the reaction has been observed. In the first group, comprising diseases of the nervous system, chronic diseases of the heart and kidneys, malignant tumors, etc., the reaction is rarely seen. When present, it usually indicates a secondary infection. The second group includes those diseases in which the reaction is almost always present, namely, typhoid fever and measles. In the diseases of the third group it is often, though not invariably, observed. Under this heading are classed scarlet fever, erysipelas, pneumonia, diphtheria, pyemia, acute miliary tuberculosis, etc. The fourth group comprises pulmonary tuberculosis, and includes acute caseous pneumonia.

For a special consideration of its occurrence in different diseases the reader is referred to Part II, notably the section on Typhoid Fever and Tuberculosis.

The reaction has been referred to the presence of alloxyproteinic acid, but this is denied by Clemens.

PLATE XXIV

As the preparation of chemically pure, crystalline diazo compounds is a difficult process, Ehrlich uses sulphanilic acid, which, when treated with nitrous acid in a nascent state, gives rise to the formation of diazo-benzene-sulphonic acid, as is shown by the equations:

1. $NaNO_2 + HCl = NaCl + HNO_2.$

2. $C_6H_4\langle^{NH_2}_{SO_3H} + HNO_2 = C_6H_4\langle^{N}_{SO_3}\rangle N + 2H_2O.$

Para-amino-benzene-sulphonic acid. Diazo-benzene-sulphonic acid.

This is the active principle in the mixture employed.

Other compounds may, of course, also be used, such as meta-amino-benzene-sulphonic acid, ortho- and para-toluidin-sulphonic acid, etc.; but of all these, Ehrlich found the common sulphanilic acid the most convenient. Two solutions, which must be kept in separate bottles, are employed. The one is a 5 per cent. solution of hydrochloric acid to which sulphanilic acid is added in the proportion of 1 gram for every 100 c.c. The other is a 0.5 per cent. solution of sodium nitrite.

The two solutions are mixed in the proportion of 40 to 1 immediately before using. A few cubic centimeters of urine are then treated with an equal volume of the reagent; the mixture is shaken and rendered alkaline with ammonium hydrate. This is best allowed to flow down the sides of the tube, so as to form a layer above the mixture. At the junction of the two fluids a colored ring will now be observed. With urines which do not contain the chromogen this will be a more or less distinct orange, while in its presence a red color is obtained. The intensity of this color may vary from eosin to a deep garnet red. If the mixture is now agitated and the reaction is positive, the foam will likewise be colored red, and upon pouring the solution into a porcelain basin containing much water a beautiful salmon color is obtained, even if only traces of the chromogen are present. Carried out in this manner no question will arise as to the presence or absence of the reaction. Ehrlich states that on standing a green sediment forms in the alkalinized mixture, and he regards this sediment as especially characteristic. My experience has been that this becomes manifest only when the color reaction is well pronounced, and I am inclined to attach more importance to the salmon color obtained upon copious dilution. With normal urines this is never obtained, and it can still be seen when inspection of the fluid in the test-tube would leave in doubt.

The older method of Ehrlich I have abandoned, as the test just described is simpler, and, in my experience, just as reliable. He advised the addition of about 50 c.c. of absolute alcohol to 10 c.c. of urine, subsequent filtration, and examination of the filtrate, as just described.

Green states that if 1 part of the sodium nitrite solution is added to 100 instead of 40 parts of the sulphanilic acid solution, a positive

reaction is no longer obtained in cases of croupous pneumonia and of pulmonary tuberculosis, while in typhoid fever the reaction occurs with the same intensity.

While in the absence of the chromogen, as I have already stated, a more or less pronounced orange color is usually obtained, exceptions have been noted. Ehrlich thus records that in urines containing biliary coloring matter an intensely dark, cloudy discoloration occurs at times, which upon boiling is changed to a well-marked reddish violet. In rare instances of ulcerative endocarditis, hepatic abscess, and intermittent fever, and more commonly in pneumonia about the time of the crisis, Ehrlich further observed an intense yolk-yellow color, before the addition of the ammonia, which becomes somewhat lighter after this is added. The reaction is supposedly referable to urobilinogen (*egg-yellow reaction*).

Of interest is the observation of Burghart, that after the administration of tannic acid, gallic acid, and certain iodin preparations, Ehrlich's reaction disappears from the urine. But, as Burghart himself suggests, it is likely that this inhibitory effect is not exerted upon the diazo-forming substances, but upon the reagents employed. Other factors, which may prevent the occurrence of Ehrlich's reaction, in pulmonary tuberculosis at least, are the occurrence of renal complications (albuminuria). Naphthalin, after its administration by the mouth, according to my experience may cause a reaction, the color of which corresponds exactly to that of the diazo reaction.

Other observers have noted a similar reaction after the administration of opium (morphine, heroin), alcohol in large amount, phenol, cresol, creosote, and guaiacol. Golden, on the other hand, denies its occurrence after the use of some of the substances mentioned.

Feri's Modification.—Feri has recently pointed out that Ehrlich's reaction may be simplified very much by rendering the urine slightly alkaline with sodium hydrate (until a turbidity develops) and then adding a small amount of a dilute solution of azophorred P. N. (paranitrodiazobenzol sulphate). This is prepared by dissolving a few granules of the dye in a test-tubeful of ordinary tap water. If the reaction is positive a bright red color develops which is imparted to the form on shaking.

Ehrlich's Dimethylaminobenzaldehyde Reaction.—Ehrlich has shown that under various pathological conditions a fine cherry-red color develops on shaking a specimen of urine with a few drops of dimethylaminobenzaldehyde in acid solution, and that the resulting pigment can be in part extracted with chloroform, and almost entirely so with epi- or dichlorhydrin. With normal urines a similar reaction can be obtained, but it is much less intense, and if done at ordinary temperatures a distinct red color does not develop. On heating, however, it appears, and can likewise be extracted with epichlorhydrin. The reaction, according to O. Neubauer, is due to urobilinogen.

As regards the occurrence of the reaction in disease I can summarize my results as follows: (1) A direct reaction, of pathological grade, does not occur in health. (2) A positive reaction is most commonly obtained in cases of tuberculosis. (3) It may also be seen in non-tubercular cases, both febrile and non-febrile. (4) It is not dependent upon the presence of the body which gives rise to the diazo reaction. (5) For its production, elevation of temperature, gastro-intestinal disturbances, and cyanosis are not essential. (6) Common to all cases seems to be an increased katabolism of the tissue albumins.

My positive results include cases of pulmonary tuberculosis, tuberculosis of the hip-joint, pneumonia, typhoid fever, appendicitis, embarras gastrique, icterus, malignant endocarditis, empyema, œsophageal carcinoma, and a remarkable instance of traumatic neurosis, in which a loss of weight of from sixty to seventy-five pounds had occurred.

My list of negative cases, on the other hand, includes, first of all, a large number of normal or supposedly normal individuals; in addition, cases of normal labor, neurasthenia, hysteria, diabetes, aortic aneurysm, myelogenous leukemia, lymphatic leukemia, acute nephritis (scarlatinal), simple diarrhea, morphinism, valvular disease, phthisis (stationary), diphtheria (before and after the use of antitoxin), typhoid fever, cases of abortion, appendicitis, influenza, chronic nephritis, cystitis, pyelitis (calculous), measles, tuberculosis of the hip-joint, cystic kidney, carcinoma of the kidney, tonsillitis, acute and chronic bronchitis, pneumonia, icterus, tubercular peritonitis, general erythema; varicocele; following various operations, such as nephrorrhaphy, removal of pus tubes, operations for vesico-vaginal fistula, fistula in ano, and suspension of the uterus. Examination of a urine containing cystin and diamins was also negative. A comparison of the negative with the positive cases will show at once that not all cases of pulmonary tuberculosis, tubercular hip-joint disease, pneumonia, typhoid fever, appendicitis, and icterus give a positive result. So far as tuberculosis is concerned, however, it appears that the reaction is more likely to occur in the actively progressive cases than in those which are more or less stationary. It was also noted that the positive cases almost all gave a positive diazo reaction, while in the negative cases this was not obtained. Exceptions, however, may also occur.

In my personal examinations I employed a 2 per cent. solution of dimethylparaminobenzaldehyde in equal parts of water and concentrated hydrochloric acid. A few cubic centimeters of urine in a test-tube are treated with from 5 to 10 drops of the reagent; the mixture is set aside or agitated for a few minutes and the color then noted. Normal urines usually turn a greenish yellow, or the normal color merely becomes intensified. At times a dark amber color develops, though this is less common in health, unless the urine is brought

to the boil before the reagent is added. In this way it is a common experience to meet with moderate or dark amber tints. With these reactions, however, I have not occupied myself, and, like Clemens and Koziczkowsky, I have only noted the reaction as positive when a distinct *cherry-red* color developed, either immediately on adding the reagent or after agitation or standing.

ACETONE

The amount of acetone which may be found in the urine under normal conditions varies between 0.008 and 0.027 gram, and is greatly influenced by the character of the diet. Whenever the carbohydrates are withdrawn the quantity rapidly increases and reaches its maximum about the seventh or eighth day. At this time from 200 to 700 mg. may be eliminated in the twenty-four hours. If, then, carbohydrates are again added to the diet, the acetonuria soon disappears. This result is not reached, however, if fats are substituted for the carbohydrates. The acetonuria is greatest when but little albuminous food and no carbohydrates at all are ingested, and during starvation the same amounts are essentially found. Increased amounts are found in fevers, the various cachexias, in conditions associated with inanition, etc. The source of the acetone in these cases was formerly sought in the increased albuminous destruction, but according to more recent research it appears that in some manner the fat metabolism is involved and that the acetonuria is the result.

Most important is the diabetic form of acetonuria (which see).

Of the febrile diseases in which acetonuria has been observed may be mentioned typhoid fever, pneumonia, scarlatina, measles, acute miliary tuberculosis, acute articular rheumatism, and septicemia. In those of short duration, on the other hand, even if the fever is high, as in acute tonsillitis, intermittent fever, the hectic fever of phthisis, etc., an increased elimination of acetone is rarely observed. In the continued fevers the acetonuria is largely referable to the character of the diet, as carbohydrates are usually excluded entirely, and I have repeatedly observed that a return to the normal occurred as soon as sugar was administered in amounts varying from 50 to 100 grams.

In certain nervous and mental diseases, as in general paresis, melancholia following epileptic seizures, and in tabes, acetonuria is frequently observed. During the second stage of general paresis increased amounts are, indeed, quite constantly found, but Hirschfeld is probably correct in stating that the psychotic form of acetonuria is largely referable to improper feeding.

A notable degree of acetonuria has been observed in connection with the pernicious vomiting of pregnancy, and in eclampsia (Baginski). A certain amount of acetone occurs normally during the

first two days of the puerperal period, but usually disappears by the third day.

According to Vicarelli acetonuria occurring in the course of pregnancy is evidence of the death of the fetus. This is possibly the rule, but exceptions have been observed.

In the primary diseases of the stomach, and notably in carcinoma, acetonuria is frequently observed, and it is possible that the acetone in these cases is, to some extent at least, formed in that organ directly.

An enterogenic form of acetonuria has further been described, and it has been urged that in these cases the acetone is referable to the formation of unusually large amounts of fatty acids. Acetonuria of this type is also observed following the ingestion of fatty acids as such (digestive form).

Acetonuria has further been observed early in the course of acute phosphorus poisoning, and may persist throughout, apparently without being an index of the severity of the case.

After chloroform narcosis the condition is also not uncommon.

Tests for Acetone.—Legal's Test.—This test may be applied to the freshly voided urine, but is not conclusive. Several cubic centimeters of urine are treated with a few drops of a strong solution of sodium nitroprusside and sodium hydrate; the mixture assumes a red color, which rapidly disappears, and in the presence of acetone is replaced by a purple or violet red when acetic acid is added. As a rule, it is better to distil the urine (500 to 1000 c.c.) after the addition of a little phosphoric acid (1 gram pro liter), and to employ the first 10 to 30 c.c. of the distillate for one or more of the following tests.

Lieben's Test.—A few cubic centimeters of the distillate are rendered strongly alkaline with caustic soda solution and treated with several drops of a dilute solution of iodopotassic iodide, when in the presence even of traces of acetone a precipitation of iodoform in crystalline form occurs. This may be recognized by its odor when the solution is heated, as also by the form of the crystals, which occur as hexagonal or stellate platelets. If traces of acetone only are present it is necessary to let the solution stand for a number of hours before examining.

Alcohol and acetic aldehyde give the same reaction. For this reason *Gunning's modification* is sometimes to be preferred, although it is not so delicate. To this end a small amount of Lugol's solution is added to the distillate and a sufficient amount of ammonia to produce a black precipitate (nitrogen iodide). This disappears on standing, and in the presence of acetone is replaced by iodoform.

Gunning's test, like that of Legal, may be tried with the native urine first.

Frommer's Test.—This test also may be applied directly to the urine, and is said to indicate the presence of 0.000001 acetone in 8 c.c. of water. It does not react with diacetic acid.

About 10 c.c. of urine are treated with about 1 gram of caustic soda in substance and—without waiting for the dissolution of the soda to occur—with 10 to 12 drops of an alcoholic solution of salicylic aldehyde (1 gram to 10 c.c. of absolute alcohol). The mixture is heated to 70° C. In the presence of acetone a marked purple-red color results at the zone of contact with the alkali.

If the alkali is added in solution the fluid first becomes yellow, later reddish, then purplish red, and finally dark carmine red. The color change occurs more rapidly on heating.

Denigès' Test (as Modified by Oppenheimer).—The reagent is prepared as follows: 20 grams of concentrated sulphuric acid are poured into 100 c.c. of distilled water, when 5 grams of freshly prepared yellow mercuric oxide are added. The mixture is allowed to stand for twenty-four hours and is then ready for use.

This reagent is added to about 3 c.c. of urine, drop by drop, until the precipitate which is thus formed no longer disappears on stirring. When this point is reached a few more drops are added. After two or three minutes the precipitate is filtered off. The clear filtrate is further treated with about 2 c.c. of the reagent and 3 to 4 c.c. of a 30 per cent. solution of sulphuric acid, and boiled for a minute or two, or, still better, placed in a vessel with boiling water. In the presence of an abundant amount of acetone a copious white precipitate forms immediately; while in the presence of traces only (less than 1 to 50,000), a slight cloud develops on standing for several minutes. The precipitate is almost entirely soluble in an excess of hydrochloric acid.

If albumin is present, the urine becomes turbid at once when the reagent is added. In that case the test is continued as described, attention being directed to the coarser precipitate which occurs later. To such urines large amounts of the reagent must be added, the idea being to precipitate everything that can be precipitated with the reagent before heating.

Oppenheimer claims that the test is as delicate as that of Lieben, viz., giving a well-pronounced reaction with a dilution of 1 to 20,000 and being still discernible with a dilution of 1 to 60,000. As diacetic acid yields acetone when treated with mineral acids, a positive result is always obtained when this is present. But as diacetic acid is usually found only in association with acetone, this fact does not lessen the value of the test, and is an error, moreover, which is common to the other tests as well.

Quantitative Estimation of Acetone.—For the purpose of estimating the amount of acetone the method of Messinger as modified by Huppert is now employed. The method does not give the acetone alone, however, but also the diacetic acid which is simultaneously present.

Principle.—The method is based upon the observation of Lieben that acetone gives rise to the formation of iodoform when treated with iodin in an alkaline solution. If then a solution of acetone is treated with a known amount of iodin it is a simple matter to determine the quantity present by retitrating the iodin which was not used in the formation of iodoform.

Solutions Required.—1. Acetic acid (50 per cent. solution).

2. Sulphuric acid (12 per cent. solution).

3. Sodium hydrate solution (50 per cent.).

4. A decinormal solution of iodine.

5. A decinormal solution of sodium thiosulphate.

6. Starch solution (see Boas' method of estimating lactic acid).

Preparation of the solutions: 1. The decinormal solution of iodin is prepared as described elsewhere (see Boas' method of estimating lactic acid).

2. As the molecular weight of sodium thiosulphate—$Na_2S_2O_3.5H_2O$—is 248, a decinormal solution of the salt would contain 24.8 grams to the liter. This quantity is dissolved in about 950 c.c. of distilled water and brought to the proper strength by titration with a decinormal solution of iodin. As 1 c.c. of the thiosulphate solution should correspond to 1 c.c. of the iodin solution, the necessary amount of water which must be added to the former is readily determined.

Method.—100 c.c. of urine, or less if much acetone is present, as determined by Legal's test, are treated with 2 c.c. of the acetic acid solution and distilled until seven-eighths of the total amount have passed over. The distillate is received in a retort which is connected with a bulb tube containing water. As soon as seven-eighths of the urine have distilled over, a small amount of the distillate of the remainder is tested for acetone according to Lieben's method. Should a positive reaction be obtained it will be necessary either to repeat the entire process with less urine, diluted to about 200 c.c., or to add about 100 c.c. of water to the residue and to distil until all the acetone has passed over. The distillate is then treated with 1 c.c. of the sulphuric acid and redistilled. The addition of the acetic acid and of the sulphuric acid respectively serves the purpose of holding back phenol and ammonia. Should the first distillate contain nitrous acid, moreover, which is recognized by the addition of a little starch paste containing a trace of potassium iodide, when the solution turns blue, the acid is removed by adding a little urea. The second distillate is received in a bottle provided with a well-ground glass stopper and holding about 1 liter. The distillate is then treated with a carefully measured quantity of the one-tenth normal solution of iodin—about 10 c.c. for 100 c.c. of urine—and sodium hydrate solution until the iodoform separates out. To this end a slight excess of the solution must be added.

Should ammonia be present, a blackish cloud will be observed at the zone of contact of the sodium hydrate and the iodin solution, and it will be necessary to repeat the entire process. The bottle is closed and shaken for about one minute. The solution is then acidified with concentrated hydrochloric acid, when the mixture assumes a brown color if iodine is present in excess. If this does not occur more of the iodine solution must be added and the process repeated until an excess is present. The excess is then retitrated with the thiosulphate solution until the fluid presents a faint yellow color. A few cubic centimeters of starch solution are now added, and the titration continued until the last trace of blue has disappeared. The number of cubic centimeters employed in the titration is finally deducted from the total amount of the iodine solution added, and the result multiplied by 0.976. The figure thus obtained indicates the amount of acetone contained in the 100 c.c. of urine, in milligrams, as 1 c.c. of the thiosulphate solution is equivalent to 1 c.c. of the iodin solution, or to 0.967 mg. of acetone.

The results as indicated express the sum of the acetone and the diacetic acid. In order to obtain the two separately, Folin has suggested the determination of both factors in one specimen of urine and the separation of acetone separately, as follows, the difference giving the diacetic acid in terms of acetone:

Folin's Method.—25 c.c. of urine are placed in an aërometer cylinder, treated with a few drops of 10 per cent. phosphoric acid, 8 to 10 grams of sodium chloride and a little coal oil. The sodium chloride is added in order to facilitate the removal of the acetone, which is insoluble in saturated NaCl solutions. The cylinder is connected with a second cylinder, as in the estimation of ammonia, an absorption tube dipping almost to the bottom, being immersed in about 150 c.c. of water plus 10 c.c. of a 40 per cent. potassium hydroxide solution, and an excess of a standardized solution of iodin. A fairly strong current of air is then run through the apparatus for 20 to 25 minutes by means of a suitable suction pump (Chapman pump). All the acetone is thus carried over and transformed into iodoform. At the expiration of twenty-five minutes the contents of the receiving cylinder are acidified with concentrated hydrochloric acid (10 c.c. for every 10 c.c. of the alkali solution) and the excess of iodin titrated back with standardized thiosulphate solution and starch as indicator. The calculation is made as described above.

Folin states that the acetone estimation can be combined with the ammonia estimation by setting up the apparatus for the ammonia first, and connecting the acetone outfit with the ammonia receiving cylinder. The air current is then adjusted for twenty to twenty-five minutes with special reference to the acetone estimation, after which the acetone receiver is disconnected and the full strength of the current is turned on for the absorption of the ammonia.

Rapidity of work is essential since the hypiodite on standing is gradually transformed into inactive iodate. This solution should hence not be prepared until everything is ready and the suction stopped after twenty-five to thirty minutes.

Another point of importance is the fact that diacetic acid may decompose spontaneously with the liberation of acetone, which likewise makes rapid work a necessity. A third point to which Folin draws attention is the importance of knowing the strength of the air current with which one has to work. This can be readily ascertained by testing a standard solution of acetone by direct titration, and then passing a given volume through the absorption apparatus. 10 c.c. of acetone diluted to 250 c.c. and 20 c.c. of this solution diluted to 500 c.c. make a suitable test solution for acetone.

An absorption tube should be used, as in the ammonia estimation.

DIACETIC ACID

The occurrence of diacetic acid in the urine is always abnormal and an indication of the existence of a certain type of acidosis. It is accordingly met with in diabetes, during starvation, in the course of various infections and certain types of gastro-intestinal disorder, following the prolonged use of anesthetics—notably chloroform, in association with the so-called pernicious vomiting of pregnancy, etc.

Tests for Diacetic Acid.—Gerhardt's Test.—To demonstrate the presence of diacetic acid a few cubic centimeters of urine are treated with a strong solution of ferric chloride drop by drop. A precipitate of phosphates is filtered off, when more of the iron solution is added to the filtrate. If a Bordeaux red color appears, this may be due to diacetic acid. To make sure, another portion of urine is boiled and similarly treated. As diacetic acid is decomposed on boiling, no reaction at all or only a faint reddish color should be obtained. As further proof a third portion of urine is acidified with sulphuric acid and extracted with ether. The diacetic acid is thus isolated. A positive reaction, when the ethereal extract is shaken with ferric chloride, will indicate the presence of diacetic acid. The color disappears on standing for twenty-four to forty-eight hours. A similar reaction is obtained with salicylic acid, antipyrin, sodium acetate, and other aromatic compounds, but the color persists for days. Sulphocyanides like diacetic acid will pass into the ethereal extract, but the color does not disappear on standing.

Arnold's Test (Modified by Lipliawski).—Two solutions are employed viz., a 1 per cent. solution of para-amino-aceto-phenone and a 1 per cent. solution of potassium nitrite. 6 c.c. of the first solution and 3 c.c. of the second are added to an equal volume of urine, together with a drop of concentrated ammonia. The mixture is shaken until

it assumes a brick-red color. From 10 drops to 2 c.c., according to the amount of diacetic acid present, are treated with 15 to 20 c.c. of concentrated hydrochloric acid (sp. gr. 1.19), 3 c.c. of chloroform, and 2 to 4 drops of an aqueous solution of ferric chloride. The tube is closed with a cork and *gently* agitated (so as to avoid emulsification), when after one-half to one minute a beautiful and very characteristic violet tinge results if diacetic acid is present. In its absence the color is yellowish or slightly reddish. The violet color persists for a long time. Bilirubin, salicylic acid, phenacetin, antipyrin, phenol, and other drugs are without disturbing influence upon the reaction. Highly colored urines should first be filtered through animal charcoal.

Allard states that both Arnold's test and that of Lipliawski give a positive result also with acetone, when this is present to the extent of more than 1 per cent.

The estimation of diacetic acid may be carried out as suggested in the section on acetone.

OXYBUTYRIC ACID

The occurrence of this acid in the urine of diabetic patients is of great clinical interest, not only from the standpoint of diagnosis but also of prognosis and treatment. Its presence may always be regarded as indicating a severe type of the disease, and when associated with marked acetonuria and diaceturia, as indicating the possible occurrence of coma. Generally speaking oxybutric acid may be encountered whenever acetone and diacetic acid are present and may indeed be much more abundant than the other two "Keto" bodies. Folin and Denis have pointed out that large amounts may be met with in obese patients when placed on starvation.

According to Herter, the condition of diabetic coma is preceded by a period of days, weeks, or months, in which there is a large excretion of β-oxybutyric acid (20 grams or more in twenty-four hours), and in which the nitrogen in the form of ammonia is largely increased. The same writer states that patients whose urines show or have shown a large excretion of organic acids are in danger of developing diabetic coma; but the nitrogen of ammonia may temporarily rise as high as 16 per cent., and yet coma may be delayed for more than seven months. The persistent excretion of more than 25 grams of β-oxybutyric acid indicates impending coma. Important also is the observation that while, as a general rule, the appearance of large amounts of organic acids is associated with the presence of much sugar, a constant relation between the two does not exist. There may thus be much sugar and little or no acid in the urine, or there may be much acid and little sugar.

Besides diabetes, the substance may be met with in scarlatina, measles, scurvy, and in starving insane patients.

The presence of oxybutyric acid may be inferred in diabetic urines, if after fermentation a rotation of the plane of polarization to the left is observed. Albumin, if present, must first be removed.

Quantitative Estimation According to Folin.—By a preliminary test with ferric chloride, one must first ascertain whether much or little oxybutyric acid is likely to be met with, as would be suggested by the intensity of the diacetic acid reaction. If this is very strong, from 25 to 50 c.c. of urine are used in the actual experiment; if the reaction is feeble, from 125 to 250 c.c. are chosen.

The volume of urine which has thus been determined is placed in a 500 c.c. volumetric flask and treated with an excess of basic acetate of lead and 10 c.c. of concentrated ammonium hydroxide. Water is added to the mark, when the mixture is shaken and filtered; 200 c.c. of the filtrate are further diluted with water to 500 to 600 c.c., treated with 15 c.c. of concentrated sulphuric acid and a little talcum (to prevent undue bumping), and then distilled until 200 to 250 c.c. of distillate have been collected (distillate A). For the distillation an 800 c.c. Kjeldahl flask is conveniently employed, which should be provided with a dropping tube so regulated that the volume of fluid does not fall below 400 c.c.

Distillate A contains the preformed acetone, and that derived from any diacetic acid that may have been present, together with volatile fatty acids. In order to eliminate these (including any formic acid which would otherwise interfere), distillate A is treated with a little fixed alkali (5 c.c. of a 10 per cent. solution of NaOH), redistilled, and the distillate A titrated with decinormal iodine and thiosulphate. (See Acetone Estimation.)

The residue of urine plus sulphuric acid from which distillate A was obtained is again distilled, while from 400 to 600 c.c. of a 0.1 to 0.5 per cent. solution of potassium bichromate are being dropped in (ordinarily 0.5 gram of the bichromate is sufficient; with much sugar or when much urine has been used even 2 or 3 grams may be required). The addition of this solution should not occur more rapidly than the distillate collects, unless the boiling mixture becomes green, which would indicate that the bichromate is being used up more rapidly. The distillation is continued until about 500 c.c. have passed over. This constitutes distillate B, which is now treated with 20 c.c. of 3 per cent. hydrogen peroxide solution and a few cubic centimeters of sodium hydroxide solution and redistilled, the idea being to oxidize any formic aldehyde which may have been formed from sugar during the chromic acid distillation, the resultant formic acid being bound by the alkali. In the final distillate, B_2 (300 c.c.), the acetone is then determined in the usual manner, with iodin and thiosulphate. This represents the acetone referable to the oxidation of the oxybutyric acid.

Folin suggests that the iodin and thiosulphate solutions be made 103.4 per cent. $\frac{n}{10}$, when 1 c.c. of this iodin solution represents 1 mg.

of acetone or 1.794 mg. of β-oxybutyric acid. The thiosulphate solution is taken as the standard and is restandardized from time to time by the $\frac{n}{10}$ iodin solution.

The ultimate results are conveniently expressed in terms of acetone. Example: Specimen of urine (400 c.c.) representing about twelve hours, from a case of puerperal eclampsia.

Acetone and diacetic acid = 0.27 gram acetone.

β-oxybutyric acid = 0.42 gram acetone.

LACTIC ACID

Sarcolactic acid is normally absent from the urine, but is met with in pathological conditions, and particularly in hepatic diseases, as the liver is normally concerned in the decomposition of lactic acid and of the lactates that have been ingested with the food. As has been pointed out, moreover, there is evidence to show that a portion of the nitrogen eliminated from the body reaches the liver as ammonium lactate, and is here transformed into urea. As a consequence, lactic acid appears in the urine whenever, as in phosphorus poisoning, acute yellow atrophy, etc., extensive destruction of the hepatic parenchyma occurs, and the formation of urea is consequently impaired. In such cases the elimination of lactic acid is associated with an increased excretion of ammonia. The same will occur when, owing to insufficient oxygenation of the blood, the power of oxidation on the part of the liver is interfered with. We accordingly find lactic acid in the urine in the chronic anemias, in cases of poisoning with carbon monoxide, in association with the various forms of circulatory and respiratory dyspnea, in cases of epilepsy immediately after the attack, following excessive muscular exercise, as in soldiers after forced marches, etc.

In order to test for lactic acid, the urine is evaporated on a water bath to a thick syrup and extracted with 95 per cent. alcohol. This is decanted off after twenty-four hours, evaporated to a syrup, acidified with dilute sulphuric acid, and extracted with ether, so long as this presents an acid reaction. The ether is then distilled off and the residue dissolved in water. This solution is treated with a few drops of a solution of basic lead acetate, filtered, the excess of lead removed by means of hydrogen sulphide, and the filtrate evaporated to dryness on a water bath, when the lactic acid will remain behind as a slightly yellowish syrup. This is then dissolved in a little water; the solution is saturated with zinc carbonate, and boiled. Zinc lactate will separate out upon evaporation, especially if a little alcohol is added, and may be recognized by the form of its crystals, viz., small prisms. These crystals are levorotatory, soluble in alcohol (1 to 1100), and contain two molecules of water of crystallization, which is lost at 105° C., so that the loss of weight after heating to this temperature must correspond to 12.9 per cent.

OXYAMYGDALIC ACID

Schultzen and Riess discovered an acid in the urine of patients who had died from acute yellow atrophy to which they gave the formula $C_8H_8O_4$. They regard it as oxyamygdalic acid and suppose it to be derived from tyrosin, which was also found, according to the equation:

$$C_9H_{11}NO_3 + 3O = CO_2 + NH_3 + C_8H_8O_4.$$

Very curiously it was not found in cases of phosphorus poisoning, but only in acute yellow atrophy. As in this disease there is coincidently with the rapid parenchymatous destruction much extravasation of blood, Nencki has suggested that the acid in question may possibly be derived from blood pigment, especially as Küster obtained from hematoporphyrin an acid which has the formula $C_8H_8O_5$, and which thus only differs from the product of Schultzen Riess by a plus of one atom of oxygen.

VOLATILE FATTY ACIDS

The term *lipaciduria* is applied to the elimination of volatile fatty acids in the urine. This occurs under normal conditions, but may be much more marked in disease. With an ordinary diet the degree of lipaciduria corresponds to from 50 to 80 c.c. $\frac{1}{10}$ normal sulphuric acid. In febrile conditions, according to v. Jaksch and Rokitansky, there is an increase which runs parallel to the height of the temperature. Rosenfeld, however, has shown that this is, strictly speaking, not correct, and that an increase is only observed in those febrile states in which resorption of breaking-down albuminous material is taking place, as in cases of tonsillar abscess, septic diphtheria putrid bronchitis, and empyema, and in general in association with all suppurative processes and hemorrhages within the body. Especially high values are found during convalescence from pneumonia during the first days following crisis. This is no doubt owing to a resorption of the exudate, and is associated with an increased elimination of nitrogen. Immediately before the crisis it is common to meet with very low values—20 c.c.—as compared with 100 to 240 c.c. during convalescence. These observations, as Rosenfeld has pointed out, may be of marked value in the diagnosis of obscure accumulations of pus.

A marked decrease in the amount of fatty acids is noted in uncomplicated cases of erysipelas and scarlatina (30 to 50 c.c.), in measles, diphtheria, and, as I have already indicated, in pneumonia preceding active resorption of the exudate (20 to 40 c.c.).

According to some observers, the amount of fatty acids in the urine may be regarded as an index of the degree of carbohydrate fermentation in the intestinal tract. Under normal conditions this may be the case, but in disease the question is probably more complicated.

The acids in question are formic acid, acetic acid, butyric acid, and propionic acid. They may be isolated as described in the chapter on the Feces.

For their *quantitative estimation* it will suffice to distil a given volume of urine with sulphuric acid and to titrate the distillate with $\frac{1}{10}$ normal sodium hydrate solution. The results are expressed in terms of the corresponding number of c.c. of $\frac{1}{10}$ normal sulphuric acid. 250 c.c. of the urine, which must be fresh or preserved with chloroform, are distilled with 50 c.c. of dilute sulphuric acid until 200 c.c. have passed over. The residue is diluted with 200 c.c. of water and the distillation continued as before. In this manner the danger that some hydrochloric acid may pass over is avoided, but it is well to make sure of this by testing with silver nitrate.

The method is exact; traces of benzoic acid are included, but in man these can be neglected.

Blumenthal mentions a case of catarrhal jaundice in which at a time when bile again flowed into the intestine a high degree of lipaciduria occurred, viz., up to 385.2 c.c. $\frac{n}{10}$ acid in lieu of the normal 50 to 80 c.c.

AMINO-ACIDS

Tyrosin, leucin, and glycocoll have long been known to occur in the urine in acute yellow atrophy and phosphorus poisoning, but aside from these conditions nothing further was known of the occurrence of amino-acids under other pathological conditions (barring cystinuria). Within recent years, however, and with more exact methods, it has been possible to show that bodies of this order may occur under the most diverse conditions. Phenylalanin, alanin, and arginin have been found in phosphorus poisoning, besides tyrosin, leucin, and glycin. Glycin, indeed, according to a recent announcement by v. Noorden, is a normal constituent of the urine and may amount to 1 per cent. of the total nitrogen output. (This is in marked contrast to the statement of Ignatowski that normal human urine only contains traces of amino-acids, at best, and that even after the subcutaneous injection of 6 grams of glycin none is demonstrable.)

Abderhalden found tyrosin in a patient dying with pneumonia, who had been suffering from arteriosclerosis, myocarditis, and diabetes. In a second case of diabetes he likewise found tyrosin and obtained a marked Millon reaction. In a third case with coma tyrosin was present also during the attack, but absent in the interim.

In a case of severe hepatic cirrhosis a marked β-naphthalin sulphochloride reaction occurred, but it was impossible to isolate amino-acids in pure form. The same observer also obtained tyrosin in a case of severe icterus, referable to complete occlusion of the common duct, and in a patient who had undergone prolonged narcosis; both urines gave a marked Millon reaction. Ignatowski found glycin constantly in the urine of 7 gouty patients; in 3 of these also other amino-acids, probably leucin and aspartic acid. In pneumonia, especially about the time of the crisis and in leukemia, he likewise obtained positive results.

Voegtlin and Barker note the occurrence of a distinct Millon reaction in the urine following the injection of tuberculin for diagnostic purposes.

In this connection the observations of Herter and Wakeman and Baldwin are of special interest. Using the method of Magnus-Levy of balancing the total bases against the total known acids, they found that in certain conditions, notably dilatation of the stomach, rheumatoid arthritis, and cirrhosis of the liver, there was a marked excess of bases over known acid equivalents. This leads to the inference that in the diseases mentioned there must have been present some other organic acid. Magnus-Levy had in this manner previously established the presence of such acids in starvation, in intestinal disturbances, phosphorus poisoning, acute yellow atrophy, and fever.

I append a few of Baldwin's results:

Apparent Excess of Acids over Bases

Average of 10 normal urines	0.2943
" in diabetes mellitus	2.96
" in rheumatoid arthritis (active stage)	0.7847
" " " "	0.5598
" " " "	0.6983
" " " "	0.6456
" " " (case 16) "	0.8377

FAT

Under strictly normal conditions the urine contains no fat, while variable amounts may be found in disease. When present in large quantities, so that it is possible to recognize it with the naked eye, the condition is termed *lipuria*. Such cases, however, are rare, and the diagnosis should only be made when it is possible to exclude accidental contamination. Smaller quantities, recognizable only with the microscope, are much more common, and are, indeed, quite constantly observed whenever fatty degeneration of the renal epithelial cells, of pus corpuscles, or of tumor particles is taking place in the urinary tract. The fat droplets may then be found floating in the

urine or attached to or embedded in any morphological elements that may be present. Lipuria may also occur when abnormally large quantities of fat are circulating in the blood. It is thus observed after the administration of cod-liver oil in large quantities, following oil inunctions, in cases of fracture of the long bones with extensive destruction of the bone marrow, in cases of eclampsia, as also in such diseases as diabetes mellitus, chronic alcoholism, phthisis, obesity, leukemia, in certain mental diseases, in affections of the pancreas and heart, etc.

The term *chyluria* or *galacturia* has been applied to a condition in which a turbid urine presenting the macroscopic appearance of milk is excreted. Upon microscopic examination it may be demonstrated that the turbidity in such cases is owing to the presence of innumerable highly refractive globules of fat, which may be removed by shaking with ether. Of morphological constituents leukocytes are occasionally encountered in large numbers. Red blood corpuscles are also seen at times, and when present in large numbers impart a rose color to the urine. Fibrinous coagula are often observed when the urine has stood for some time, and the entire bulk of urine may even become transformed into a gelatinous mass. Albumin is present in most cases in the absence of other constituents pointing to renal disease, such as tube casts and renal epithelial cells. Leucin, tyrosin, and cholesterin may also at times be found, particularly the latter. It has been quite generally accepted that chyluria is due to the presence of the Filaria sanguinis hominis; but while filarias are undoubtedly present in the blood in the majority of instances, and may also be present in the urine, it has been demonstrated that cases occur in which filariasis does not exist.

FERMENTS

Ferments may be demonstrated in every urine, both under physiological and pathological conditions. Pepsin is said to be absent in cases of typhoid fever, carcinoma of the stomach, and possibly also in nephritis. In order to demonstrate its presence, a small flake of boiled fibrin is placed in the urine, and after several hours removed to a 2 to 3 pro mille solution of hydrochloric acid. The pepsin, if present, will be deposited upon the fibrin and effect digestion of the latter in the hydrochloric acid solution.

Diastase, a milk-curdling ferment, and a fat-splitting ferment have also been observed. It is noteworthy that the fat-splitting ferment was first encountered in a case of hemorrhagic pancreatitis, and it has been suggested that its presence may possibly be of value in the diagnosis of the disease. Opie, who reports the case, demonstrated its presence by the method of Kastle and Loevenhart. Only a small amount of urine was obtained. This was neutralized with $\frac{n}{10}$ alkali

and divided into two portions. To one portion were added 0.25 c.c. of ethyl butyrate, together with a small quantity of litmus solution and 0.1 c.c. of toluol. The second portion, used as a control, was boiled in order to destroy the ferment if present, and ethyl butyrate added. Both specimens were kept at 37° C.; at the end of twenty-four hours the unboiled specimen had acquired a well-marked acid reaction, while the control specimen was little if at all changed. A quantitative estimation can be made by titrating the two specimens with $\frac{n}{10}$ alkali (using phenolphthalein as an indicator), and noting the amount of ethyl butyrate which is split by the ferment. The titration should be made after adding to each specimen 0.5 c.c. more of $\frac{n}{10}$ HCl than of the $\frac{n}{10}$ alkali originally used, and to shake out the butyric acid with 50 c.c. of ether and 25 c.c. of alcohol; the acid is then titrated directly in the ethereal solution.

Since the diagnosis of acute lesions of the pancreas is difficult and at times impossible the demonstration of the constant occurrence of the ferment under such circumstances would be of great importance. Its diagnostic importance has been further emphasized by the experimental work of Hewlett on dogs (which see).

GASES

Every urine contains a small amount of gases, notably carbon dioxide, oxygen, and nitrogen, which may be withdrawn by means of an air pump.

Under pathological conditions hydrogen sulphide is at times also found, constituting the condition known as *hydrothionuria.* In some instances this is referable to a diffusion of the gas into the bladder from neighboring organs or accumulations of pus; but this is rare. In others an abscess has ruptured into the bladder, or a direct communication exists between it and the bowel. Under such conditions it can, of course, not be surprising that hydrogen sulphide together with other products of albuminous putrefaction are eliminated in the urine. More commonly, however, the hydrothionuria occurs idiopathically, and is then referable to the action of certain microörganisms. This can be readily demonstrated by adding a few cubic centimeters of such urine to normal urine, when upon standing the formation of hydrogen sulphide may be demonstrated in the normal specimen. The common organisms, however, which cause ammoniacal decomposition apparently have no part in this process, and the formation of the hydrogen sulphide may be observed before ammoniacal decomposition has set in, and while the reaction is yet acid. If a small amount of ordinary decomposing urine, moreover, is added to fresh normal urine, no hydrogen sulphide is, as a rule, produced. The character of the organisms in question is variable; sometimes micrococci are found, at other times

bacilli, and in still other instances both. Besides being capable of producing hydrogen sulphide from the sulphur bodies of the urine, some of them also cause the formation of ammonium carbonate in dilute solutions of urea.

The source of the hydrogen sulphide in cases of hydrothionuria is in most cases probably the so-called neutral sulphur, but it is possible that the oxidized sulphur is at times also attacked. In cystinuria, in which the neutral sulphur is more or less increased, hydrothionuria is commonly observed. Its occurrence in such cases is indeed so frequent that I am inclined to suspect cystinuria, even though crystals of cystin are not found in the sediment.

In a few recorded instances the hydrothionuria accompanied indigosuria, viz., the presence of free indigo blue in the urine; and this Müller has likewise shown to be referable to the action of certain microörganisms. (See Indigosuria.)

The formation of hydrogen sulphide in decomposing urines containing albumin is, of course, common, and should not be confused with the idiopathic hydrothionuria here described.

The chemical test for hydrogen sulphide is very simple. A strip of filter paper is moistened with a few drops of sodium hydrate and lead acetate solution and clamped into the neck of the bottle containing the urine. After a variable length of time, in some instances immediately, in others only after twelve to twenty-four hours, a discoloration of the paper will be observed, varying from a grayish brown to black, according to the amount present. When this is large it is, of course, also recognized by its characteristic odor.

PTOMAINS

The only substances belonging to the class of ptomains which have thus far been obtained from the urine in amounts sufficient to establish their identity are *cadaverin* and *putrescin*. They were originally discovered by Brieger in putrefying cadavers, and subsequently also found in cultures of the bacillus of Asiatic cholera, the Finkler-Prior bacillus of cholerina, the bacillus of tetanus, and in the rice-water stools of cholera patients. From the urine, cadaverin, putrescin, and a third diamin isomeric with cadaverin, which has been regarded as saprin or neuridin, were first obtained by Baumann and v. Udranszky in a case of cystinuria, and it appears that diaminuria occurs only in association with this disease. All attempts to isolate diamins from the urine under other pathological conditions at least have given rise to negative results. Regarding the origin of the ptomains in question there can be no doubt, I think, that they are derived from the corresponding hexon bases, arginin and lysin, as the result of a definite metabolic anomaly, of which the cystinuria is also one expression. I have advocated this view for some years, and Löwy

and Neuberg have recently furnished the experimental proof for this supposition. They found in a cystinuric individual who was not excreting any diamins that putrescin and cadaverin appeared when the corresponding hexon bases were ingested. Löwy and Neuberg further claim to have found tyrosin and aspartic acid when these were given by the mouth, which would tend to show that in the cystinuric there is even a more extensive inability to oxidize amino-acids than the cystinuria and diaminuria alone would indicate. I have not been able to verify these findings, however, so far as tyrosin is concerned, and Folin also obtained negative results.

Putrescin has been found by Baumann and v. Udranszky, Bödtker, and Garrod. Brieger, Stadthagen, Leo, Garrod, Lewis, and I have succeeded in isolating cadaverin from such urines. Others have been less successful. As regards the question whether diaminuria and cystinuria invariably co-exist, I have shown that this is not always so, and that the two conditions may alternate, and that the one may temporarily disappear while the other continues. Whether or not cases occur in which diamins are constantly absent I am not prepared to say. Cases have been reported by Garrod and others in which no diamins could be found, but it is possible that our analytical methods are not sufficiently delicate to demonstrate mere traces.

The amount of diamins which may be met with in the urine of cystinuric patients is extremely variable. In one case I was able to isolate 1.6 grams of the benzoylated cadaverin from the collected urine of twenty-four hours. On other days traces only were present, and at times no diamins at all could be found. In the case of Dr. Lewis, I obtained only 0.3 gram from 12,000 c.c.

Isolation of Diamins.—Method of Baumann and v. Udranszky.—The collected urine of at least twenty-four hours is shaken with a 10 per cent. solution of sodium hydrate and benzoyl chloride in the proportion of 1500 to 200 to 25 until the odor of the benzoyl chloride has entirely disappeared. The resulting precipitate contains phosphates, the benzoyl compounds of the normal carbohydrates of the urine, and a portion of the benzoylated diamins. These are filtered off with the aid of a suction pump and digested with alcohol. The filtered alcoholic extract is concentrated to a small volume and poured into about 30 times its amount of water. Upon standing for from twelve to forty-eight hours the benzoylated diamins separate out in the milky fluid in the form of a more or less voluminous sediment composed of fine, intensely white crystals. In order to remove the benzoylated carbohydrates likewise present, the precipitate is redissolved in alcohol, the solution concentrated to a small volume, and diluted with water as described. This process is repeated several times. The resulting crystals, if both diamins are present, will lose their water of crystallization at 120° C. and melt at 140° C.

A smaller portion of the benzoylated diamins remains in the first

filtrate. In order to recover this the filtrate is acidified with sulphuric acid and extracted with ether. The ethereal residue, before congealing, is placed in as much of a 12 per cent. solution of sodium hydrate as is required for its neutralization, when from 3 to 4 times the volume of the same solution is added. This mixture is placed in the cold, when long needles and platelets separate out, which consist of the sodium compound of benzoyl cystin and the benzoylated diamins. The sediment is filtered off and placed in cold water, in which the sodium-benzoyl cystin dissolves, while the benzoylated diamins remain undissolved.

In order to separate the putrescin from the cadaverin. the crystals are dissolved in a little warm alcohol and treated with 20 times the volume of ether. Benzoyl putrescin is thus thrown down, and may be recognized by its melting point, viz., 175° to 176° C., while the ethereal residue contains the benzoyl cadaverin, which melts at from 129° to 130° C.

The diamins may then be separated from the benzoyl radicle by heating the crystals on a water bath with a mixture of equal parts of alcohol and concentrated hydrochloric acid until a specimen is entirely dissolved by sodium hydrate. The separation is complete after from twenty-four to forty-eight hours, according to the amount present. The solution is then diluted with water, when the benzoic acid, which has been formed, separates out and is filtered off. After extracting with ether, in order to remove any benzoic acid still remaining, the filtrate is evaporated to dryness. A crystalline mass remains which is easily soluble in water, but with difficulty in alcohol. This consists of putrescin and cadaverin hydrochlorates, from which the various double salts with platinum, silver, mercury, etc., can be readily obtained. The platinum salt of cadaverin is formed by adding an alcoholic solution of platinum chloride to a solution of the hydrochlorate in alcohol; it occurs as a voluminous yellow, crystalline mass, which can be purified by recrystallization from hot water. When this salt is decomposed by hydrogen sulphide the hydrochlorate again results, from which the free base is obtained by distillation with caustic potash. During this distillation water passes over at first; and above 160° C. a colorless oil appears, the boiling point of which is about 173° C. This constitutes the free base, which may be identified by its sperm-like odor and the avidity with which it attracts carbon dioxide from the air to form carbonate.

DETERMINATION OF RENAL INSUFFICIENCY

The Phenolsulphonephthalein Test of Rowntree and Geraghty (*Permeation test*).—This test, which has been introduced by Rowntree and Geraghty, seems to be the most satisfactory of the various so-called functional tests. It is conducted as follows: Twenty to

thirty minutes before the examination is begun the patient receives 200 to 400 c.c. of water, so as to insure a sufficient diuresis, as otherwise a delay in the time of the appearance of the drug may be due to lack of secretion. The patient is then instructed to empty his bladder, and now receives 6 mgms. of the phthalein,[1] either hypodermically, intravenously, or intramuscularly, the latter being the route of preference (lumbar muscles).

The time of the injection is noted and the patient told to pass his urine at the end of one hour and ten minutes, and again at the end of two hours and ten minutes, counting from the time of injection, the extra ten minutes being allowed for the usual time that it takes the drug to appear in the urine. The two specimens are kept separate and rendered distinctly acid (with phosphoric acid), if a longer period than a few hours must elapse before the examination is made; otherwise they are sent to the laboratory as they are.

In cases of urinary obstruction the patient's bladder is first emptied by catheter which is then allowed to remain in position throughout the test. In this case the time of the drug's first appearance may be determined by placing a drop of a strong solution (25 per cent.) of sodium hydrate solution in a receiving test-tube, and noting the time when the first trace of pink appears. The catheter is then corked, and the bladder emptied after one hour, counting from the first appearance of the phthalein.[2]

When the test is to be used in conjunction with ureteral catheterization, Geraghty recommends that the injection be made intravenously and that the urine be collected for two separate fifteen-minute intervals, the time of collection beginning with the appearance of the drug on the first side. To obtain the best results, however, an intramuscular injection should be made, followed by a collection of the urine for one hour, so as to eliminate any chance of variable elimination which is possible in short intervals; this, moreover, is to be supplemented by the usual routine examination, for purposes of comparison and to check up the amount of catheter inhibition, if such should occur.

The next step then is to ascertain how much of the drug has been excreted during the two periods of collection. To this end both lots (A and B) are (separately) rendered strongly alkaline with an amount of 25 per cent. caustic soda solution sufficient to bring out the maximum of color (a fine purplish red), diluted to 1000 c.c.

[1] The solution is prepared as follows: 0.6 gram of the substance and 0.84 c.c. of double normal sodium hydrate solution are diluted to 100 c.c. with 0.75 per cent. saline solution, when 0.15 c.c. more of the double normal NaOH solution is added. The resultant product is filtered and contains 0.6 mg. to the c.c. This may be procured, in little ampoules ready for use, from Hynson & Westcott, of Baltimore, who also market the colorimeter referred to below.

[2] In cases where the catheter is to be employed it is recommended to previously place the patient under hexamethylenetetramin.

with clear water and filtered. The amount of phenolsulphonephthalein is then determined colorimetrically, either by the aid of a Duboscq instrument or the cheaper modification of the Hellige colorimeter devised by Rowntree and Geraghty (see Fig. 162). In the case of the latter the wedge-shaped cup *A* is filled with the standard alkaline solution (6 mg. per liter alkalinized with one or two drops of the 25 per cent. solution of NaOH). The alkalinized and diluted urine is poured into the rectangular cup *B*, when the wedge-shaped cup is manipulated by means of the screw *C* until

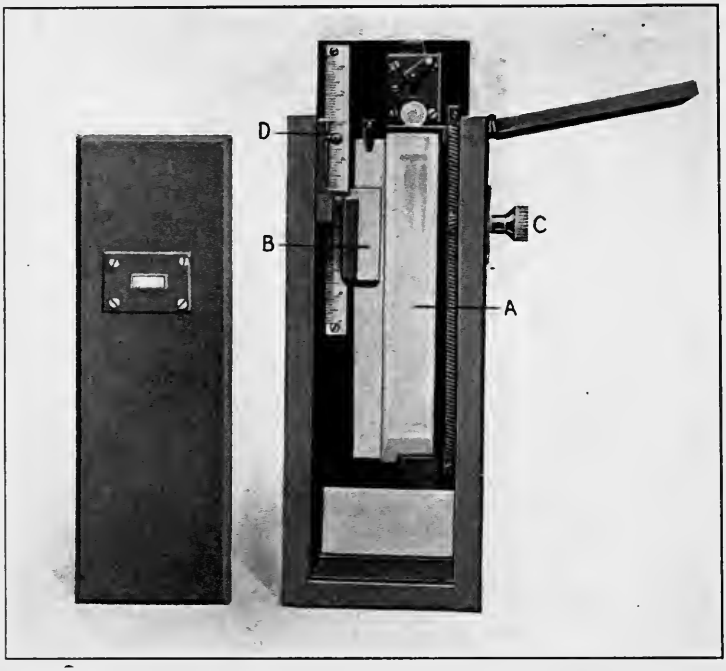


Fig. 162.—Hellige's colorimeter.

the two sides of the color field are identical in intensity. The percentage is then read directly by the position of the indicator on the scale *D*. If after adding the alkali the coloration of the urine is slight, showing a small excretion of the phthalein, the dilution should only be carried to 250 or 500 c.c. and the readings on the scale divided by 4 or 2, as the case may be.

If a Duboscq instrument is available the comparison is made with a one-half strength standard solution of the drug (3 mg. to the liter, alkalinized with one or two drops of the 25 per cent. NaOH solution). The one cup of the instrument is then about half filled with this

solution and adjusted at the ten mark. The other cup is similarly filled, the color equalized and the reading taken, any fraction indicated by the Vernier being taken into account. The calculation is made as shown in the following example, the second reading having been 20. As the amount of substance is inversely proportionate to the depth of the column of fluid, the proportion $10 : 20 : : x : 100$, *i. e.*, $x = \frac{10 \times 100}{20} = 50$ per cent., indicates the amount of the substance eliminated as compared with the standard color solution, containing 3 mg. to the liter. But as 6 mg. were injected, we must still ascertain what percentage of this amount that just found represents. In the example given, 50 per cent. would represent 1.5 mg. The actual percentage eliminated would now be found according to the equation $6 \text{ mg.} : 100 : : 1.5 : x$, which would give 25 per cent.

In the absence of a colorimeter fairly satisfactory results may be obtained by placing a measured quantity of the alkalinized urine (diluted or undiluted according to the intensity of the color) in a test-tube and comparing the color with a tube containing a known quantity of the drug, and diluting the latter until the colors match, when the percentage eliminated is ascertained by simple calculation.

Results.—Under normal conditions the time of the appearance of the drug varies between five and eleven minutes, the elimination of the first hour between 40 and 60 per cent., and that of the second hour between 20 and 25 per cent., so that from 60 to 85 per cent. have usually been eliminated by the end of the experiment. In diseases affecting the integrity of the renal epithelium, on the other hand, there is evidence of an impeded elimination, and this is *cæteris paribus* the more marked the greater the degree of tubular involvement.

Of five cases of acute nephritis reported by Rowntree and Geraghty, the permeability of the kidneys to the phthalein was found diminished when the condition was considered clinically as grave. Of twenty-five cases of so-called parenchymatous nephritis five were very mild and of short duration; in these the time of the appearance of the drug and the amount excreted was normal. In the other cases in which the disease was of ordinary severity and of longer standing the time of the appearance was always delayed (ten to twenty-five minutes) and the amount excreted definitely below normal, but varying with the condition of the patient, the lowest values being found in those cases where the disease was of long standing and associated with sclerotic changes. In some instances, indeed, no trace of the drug could be found.

In twenty-three cases of chronic interstitial nephritis the appearance of the drug was usually markedly delayed and the output much diminished. In these cases especially the value of the test was apparent, as the clinical condition frequently did not permit an insight into the degree of renal destruction, while the correctness of

the information conveyed by the test was often shown in a striking manner by the subsequent course of the malady.

Especially interesting are the results obtained in cases of uremia. Twenty-five cases were examined. In sixteen of these the condition was grave, and of these in turn eleven died during the attack. In all of these the phthalein elimination was zero or a faint trace only for the two hours. Five of the sixteen recovered from the attack; in these the elimination varied between 13 and 20 per cent. In mild cases of chronic uremia very low values also were found (trace to 10 per cent.); four of these died within four months. Even in cases presenting no uremic symptoms a low output justified a dire prognosis.

So far as the present evidence goes it would seem that by means of the phthalein test one can also distinguish between those cases of cardiac disease with broken compensation in which the kidneys have not been permanently damaged, and those cases in which cardiac insufficiency is associated with varying grades of true nephritis.

In *fine* I would emphasize the value of the test in surgical practice and particularly in connection with operations on the prostate. In cases of enlarged prostate a marked decrease in the amount eliminated almost invariably means severe derangement of renal function which may be either of temporary or permanent character. Under such conditions one should proceed with extreme caution and no surgical intervention should be attempted without further study together with preliminary treatment. Under this regime repeated tests will demonstrate eventually the nature of the derangement, for in true interstitial nephritis the output will continue low, whereas, if the derangement is purely functional or secondary to pyelonephritis, improvement will usually follow as a result of the treatment, and will be indicated by a decrease in the time of the appearance of the drug and simultaneously an increase in the amount eliminated. The functional derangement due to infection in these cases is a much more dangerous condition than is the presence of even a fairly advanced condition of interstitial nephritis. The use of the test enables one to select a favorable time for operation. In cases exhibiting a continued suspiciously low output, the use of nitrous oxide gas is suggested as preferable to ether, in order to protect the kidneys. When only a trace of the drug continues to be excreted, operation should not be attempted at all except in an emergency, even though the patient presents no symptoms of uremia.

Regarding the value of the phthalein test in the study of unilateral and bilateral disease of the kidney, Rowntree and Geraghty remark the following: In normal cases the time of the appearance of the drug from the two sides has been almost always the same and in the majority of cases this has been five to ten minutes following sub-

cutaneous and three to five minutes following intravenous injection. The time of appearance, of course, will vary somewhat with the rate of urinary secretion. Normally the amount excreted by each kidney will be practically the same.

When one kidney only is diseased the time of the appearance of the drug is delayed on the diseased side, and the amount excreted is not only relatively but absolutely decreased. The amount of delay in the time of appearance is comparatively of little value. Reliance is to be placed only on the quantity excreted during a period of one-quarter, one-half, or one hour, depending on the method of administration.

Although in the majority of the cases of unilateral disease the combined output is equal to that of two normal kidneys, the greater part of the excretion is shown to be performed by the healthy kidney. In proportion to the decrease in function on the diseased side approximately, there is a proportionate increase in the function on the healthy side. In such cases following nephrectomy the remaining kidney eliminates, after the lapse of two or three weeks, an amount of the drug which is normally excreted by two healthy kidneys. In all cases studied, the output from the remaining kidney has been greater than the combined output from the two kidneys prior to operation.

In bilateral disease it has been found possible to determine the individual function (absolute or relative) of each kidney. It is in this class of cases particularly that the shortcomings of other functional tests have been most apparent, as one kidney may be doing twice or three times the amount of work of the opposite kidney, and still be unable to assume the additional work of the other kidney. It may be doing the major part of the work at the expense of all or nearly all of its reserve power, but the phthalein test determines whether the kidney has a functional capacity which is normal, less than or greater than normal and to what degree.

Since the introduction of this test, only a few years ago, the claims made by its originators have been so extensively confirmed that the divergent results obtained by others must be attributed to accidental factors. As it is it ranks in diagnostic and prognostic value with the most important tests which are made in the clinical laboratory.

MICROSCOPIC EXAMINATION OF THE URINE

In the chapter treating of the general physical characteristics of the urine it was stated that, on standing, every urine gradually becomes cloudy owing to the development of the so-called nubecula. This was shown to consist of a few leukocytes, a small number of pavement epithelial cells derived from the urinary and genital passages, and, under certain conditions, of a few crystals of uric acid,

of calcium oxalate, or of both. It was further pointed out that owing to a diminution in the acidity of the urine on standing, in consequence of an interaction between the neutral sodium urate and the acid sodium phosphate, a sediment is thrown down which consists of acid sodium urate, and at times of free uric acid. (See Reaction.) Should the reaction of the urine be alkaline, however, when freshly voided, a condition which may occur physiologically, when it is dependent upon the ingestion of large quantities of vegetables rich in organic salts of the alkalies, but which may also be due to ammoniacal decomposition, those constituents of the urine which are held in solution merely in consequence of the presence of acid sodium phosphate are also thrown down. In that case the sediment consists essentially of calcium, magnesium, and ammonium salts. Crystals of ammoniomagnesium phosphate, it is true, may also be observed in alkaline urines of the first variety, but they are then almost always due to an increased elimination of ammonia, and hence are rarely observed under physiological conditions.

Normally calcium is found only in combination with phosphoric acid and carbonic acid. Of the three possible calcium salts of phosphoric acid—*i. e.*, $Ca_3(PO_4)_2$, $CaHPO_4$, and $Ca(H_2PO_4)_2$—only the first two are found in an alkaline urine, but they may also be observed in specimens which are either neutral or but faintly acid. The acid calcium phosphate, $Ca(H_2PO_4)_2$, is seen but rarely in sediments; it is precipitated together with uric acid and under similar conditions. Calcium carbonate, $CaCO_3$, is seen only in neutral or alkaline urines. As soon as ammoniacal fermentation has begun, ammonium salts are formed, viz., ammonium urate and ammoniomagnesium phosphate.

The following table shows the various mineral constituents usually observed in sediments, the reaction of the urine being in every case the all-important factor:

Reaction acid:
- Uric acid.
- Sodium urate.
- Calcium oxalate.
- Primary calcium phosphate.
- Ammoniomagnesium phosphate

Reaction alkaline (referable to fixed alkalies):
- Secondary calcium phosphate.
- Tricalcium phosphate.
- Calcium carbonate.
- Ammoniomagnesium phosphate.

Reaction alkaline (referable to ammonia):
- Ammonium urate.
- Ammoniomagnesium phosphate.
- Tricalcium phosphate.
- Calcium carbonate.

In pathological conditions still other unorganized substances may be observed, viz., cystin, xanthin, hippuric acid, indigo, urorubin, hematoidin, magnesium phosphate, calcium sulphate, cholesterin, leucin, tyrosin, fats, soaps of magnesium and calcium, etc. Of these, cystin, xanthin, hippuric acid, tyrosin, calcium sulphate, hematoidin, magnesium phosphate, leucin, and the soaps of magnesium and calcium occur principally in acid urines, while indigo, urorubin, and cholesterin are usually only found in alkaline specimens. Before considering these various constituents in detail, a few words regarding sediments in general and the method to be followed in their microscopic examination may not be out of place.

An idea of the nature of a deposit may often be formed by simple inspection, especially if the reaction of the urine is known.

A crystalline sediment, presenting a brick-red color and appearing to the naked eye like cayenne pepper, is referable to uric acid. On the other hand, a salmon-red, amorphous deposit occurring in an acid urine consists essentially of sodium urate. Should doubt be felt, it will only be necessary to heat the urine, when the urate deposit will dissolve. A white, flocculent sediment in an alkaline urine is usually referable to a mixture of phosphates and carbonates, and will dissolve upon the addition of acetic acid, but remains unaffected by heat.

A sediment consisting of pus, and occurring in alkaline urines, is frequently mistaken for a phosphatic deposit by the beginner. Aside from a microscopic examination, the question may be settled by the addition of a small piece of caustic soda and stirring, when in the presence of pus the liquid becomes mucilaginous and ropy. If much pus is present, a tough, jelly-like mass will be formed, which escapes from the vessel *en masse* when the urine is poured out. Such a sediment, moreover, does not disappear upon the addition of an acid, and is rendered still more dense upon the application of heat.

Blood when present beyond traces may also be recognized.

As a general rule, the non-organized elements of a sediment are of little clinical interest.

Students are frequently in the habit of diagnosticating an excessive normal or subnormal elimination of one or another urinary constituent from the result of a microscopic examination. This is unwarrantable. It should always be remembered that no conclusions whatsoever can be drawn in this manner as to the amount actually eliminated. Nothing would be more erroneous than to infer an excessive excretion, not to speak of an excessive production of uric acid or of oxalic acid from the fact that crystals of these substances are seen in large numbers under the microscope. Again and again cases are observed in which an excessive elimination of uric acid, oxalic acid, or phosphates is thus diagnosticated in which

chemical analysis shows not only no increase but even a diminution of the normal quantity.

A urine which is turbid when passed may be examined microscopically at once. As a rule, however, it is necessary to wait until a sediment has formed. To this end the urine should be kept in a clean and well-stoppered bottle. A small amount of chloroform is added if necessary, and will preserve the specimen almost indefinitely. A few drops of sediment are then removed by means of a *clean* pipette, carried down to the sediment, with the distal end tightly closed by the finger, care being taken not to allow the urine to *rush* into the tube by suddenly releasing the pressure, but withdrawing an amount just sufficient for an examination. This is then spread over a *clean slide* that has been moistened by the breath, when the specimen may be examined at once. *Covering the specimen with a slip is unnecessary. A low power of the microscope* (B. & L. $\frac{2}{3}$; Leitz 3) *should always be employed, and the high power only used to study details of structure.*

If a centrifugal machine is available, it is, of course, not necessary to let the urine stand until a sediment has formed. An amount sufficient for a microscopic examination can then be obtained in a few minutes.

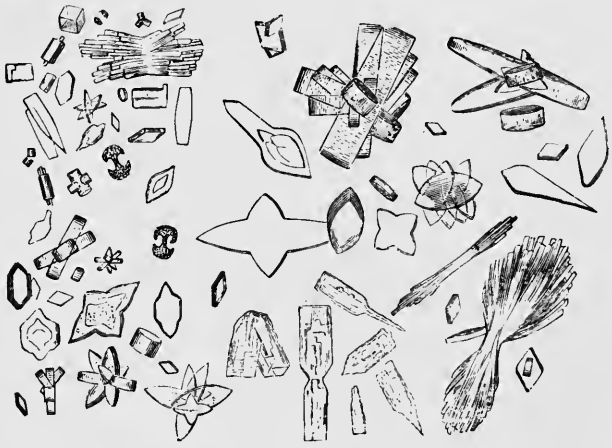


Fig. 163.—Various forms of uric acid crystals. (Finlayson.)

NON-ORGANIZED SEDIMENTS

Sediments Occurring in Acid Urines.—Uric Acid.—The form which uric acid crystals may present in a deposit varies greatly, the most common being the so-called whetstone form (Figs. 163 and 164). The crystals may occur singly or in groups. Accidental impurities, such as threads or hairs, are at times covered with such crystals, forming long cylinders. Very frequently uric acid crystallizes in the form

of large rosettes of drawn-out whetstone crystals, presenting a brownish-red color, referable to uroerythrin, when they are often visible to the naked eye, and form the well-known *brick-dust sediment.* While it is generally stated that uric acid crystals can always be recognized by their color, which may vary from a light yellow to a dark brown, this is, in my experience, not the case. I have often

Fig. 164.—Uric acid crystals.

seen uric acid sediments in which the crystals formed small rhombic plates with rounded edges, and were absolutely devoid of coloring matter, so far as a microscopic examination could show. Uric acid "dumb-bells" are also at times observed, and may be mistaken for calcium oxalate. Hexagonal plates of uric acid have been similarly confounded with cystin.

A uric acid sediment may be observed in cases in which an increased excretion of uric acid occurs, but it does not follow that a

uric acid sediment indicates an increased elimination. Such an assumption would only be warrantable if a quantitative estimation had been made. It is more common to meet with uric acid sediments where the actual amount is not increased than the contrary. Brick-dust sediments are frequently observed during cold weather, but it would be erroneous to infer an increased elimination from such an occurrence, as the phenomenon is owing to the fact that uric acid is less soluble in cold than in warm water. During the summer months, for the same reason, a deposit of uric acid is less frequently observed, although an increased amount may nevertheless be present, being held in solution owing to the higher temperature. The more concentrated the urine, the more readily will such a deposit form. It is hence noted after profuse perspiration, following severe muscular exercise, in acute rheumatism with copious diaphoresis, in acute gastritis and enteritis associated with copious vomiting or diarrhea, during the crisis of pneumonia (particularly if accompanied by much sweating), etc.

Where a distinct tendency exists for the separation of uric acid sediments, as in cases where the urine is habitually overacid, the possibility of the same occurrence within the urinary passages should be borne in mind (gravel, calculus). F. Müller has recently shown that the habitual separation of uric acid from the urine which is noted in such cases and which is commonly associated with vague dyspeptic and nervous disturbances is referable to the presence in such urines of considerable amounts of organic acids of unknown composition.

Chemically, the nature of a uric acid sediment may be recognized by the fact that the crystals dissolve upon the addition of sodium hydrate and reappear in the rhombic form upon acidifying with hydrochloric acid. When heated with dilute nitric acid the beautiful red color of ammonium purpurate is obtained upon the subsequent addition of ammonia (murexide test), as described elsewhere.

Amorphous Urates.—Sodium and potassium urates frequently, and especially in fevers, form sediments of such density that upon microscopic examination it is almost impossible to discern anything but innumerable amorphous granules scattered over the entire field and obscuring all other elements that may be present. Cells or casts will frequently be seen studded with these granules. In such cases it is best to heat the urine to a temperature of 50° C., and to centrifugalize the urine as soon as it has cleared.

Urate sediments are always colored, the tint varying from a dirty yellowish brown to a bright salmon red, owing to the presence of uroerythrin. Difficulties can hence never arise in determining the nature of the sediment, as a colored deposit appearing in an acid urine which dissolves upon the application of heat cannot be due to anything but urates. If a drop of the sediment, moreover, is treated upon a slide with a drop of hydrochloric acid, characteristic whet-

stone crystals of uric acid separate out, but the greater portion appears in the form of rhombic platelets.

Calcium Oxalate.—This substance generally appears in urinary sediments in the form of colorless, highly refractive octahedra (Fig. 165), which vary greatly in size; some appear as mere specks under even a comparatively high power, while others may attain the dimensions of a large leukocyte. Frequently one axis is longer than the other. From the fact that their diagonal planes are highly refractive, apparently dividing the superficial plane into four triangles, they have been compared to envelopes, and it is this envelope form of the crystals which is especially characteristic. In the same specimen of urine so-called dumb-bell forms may be seen, which appear to be made up of two bundles of needle-like crystals united in the form of the figure 8. Other forms may also be seen, and are shown in the accompanying figure (Fig. 166).

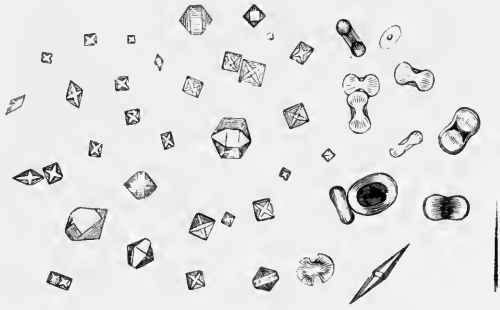


FIG. 165.—Calcium oxalate crystals. (Finlayson.)

FIG. 166.—Calcium oxalate crystals

While the envelope crystals are highly characteristic, and can hardly be mistaken for any other substance, the student may at times confound them with crystals of ammoniomagnesium phosphate. This error may be avoided if it is remembered that the calcium oxalate crystals are usually not so large as those of the magnesium salt, and that the latter dissolve upon the addition of acetic acid, in which calcium oxalate is insoluble. The distinction from uric acid, if we are dealing with the dumb-bell form, cannot always be made by mere inspection. A drop of caustic soda should be added, which will dissolve the crystals if they are uric acid, while calcium oxalate remains unchanged.

It has been pointed out that under strictly normal conditions a few isolated crystals of calcium oxalate may be found in the primitive nubecula, so that their presence in urinary sediments cannot be regarded as pathological. After the ingestion of certain vegetables and fruits, notably tomatoes, rhubarb, garlic, asparagus, and oranges, or following the continued administration of sodium bicarbonate or the salts of vegetable acids, calcium oxalate crystals may be observed

in large numbers; so also in certain diseases, such as diabetes mellitus, catarrhal jaundice, phthisis, emphysema, etc.

As in the case of uric acid, no inference as to the quantity eliminated can be drawn from a microscopic examination of the sediment. The frequent occurrence of abundant sediments of this substance may, however, generally be regarded as abnormal, providing that such an occurrence cannot be explained by the nature of the diet. It is very suggestive to note the frequency with which such sediments are observed in cases of neurasthenia associated with a mild degree of albuminuria, as also in various digestive neuroses. Finally, as with uric acid, the possibility of the formation of renal calculi should be borne in mind whenever abundant sediments of calcium oxalate are encountered upon frequent examinations.

Monocalcium Phosphate.—Crystals of this salt are rarely seen, and only in specimens presenting a highly acid reaction, when uric acid crystals are also frequently observed in large numbers (Fig. 167). They are colorless and soluble in acetic acid.

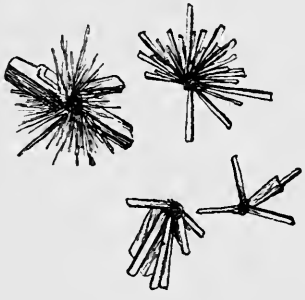


Fig. 167.—Monocalcium phosphate crystals.

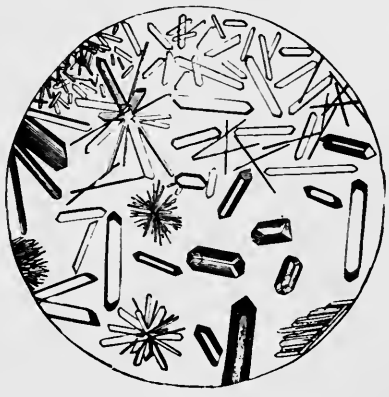


Fig. 168.—Hippuric acid crystals

Hippuric Acid.—Hippuric acid crystals (Fig. 168) have been observed, although rarely, in urinary sediments, in acute febrile diseases, diabetes, and chorea, while their occurrence following the ingestion of large amounts of prunes, mulberries, blueberries, or the administration of benzoic acid and salicylic acid, is more common.

Hippuric acid occurs in the form of fine needles or rhombic prisms and columns, the ends of which terminate in two or four planes, at times resembling the crystals of ammoniomagnesium phosphate and of uric acid. From the former they may be readily distinguished by their insolubility in hydrochloric acid, and from the latter by the fact that they do not give the murexide reaction when treated with nitric acid and ammonia. In the case of urines rich in hippuric acid in which the substance does not appear in the sediment, it is

well to add a small amount of hydrochloric acid, when the crystals will gradually separate out. Their presence does not appear to possess any clinical significance.

Calcium Sulphate.—Calcium sulphate, in the form of long, colorless needles or elongated prismatic tablets (Fig. 169), has been observed in urinary sediments in only two cases. In both the urine, especially on standing, deposited a milky looking sediment, the reaction being strongly acid. It may be recognized by its insolubility in acids and ammonia.

Cystin.—This is rarely seen in urinary sediments. It occurs in the form of colorless, hexagonal platelets, which are very characteristic (Fig. 170). The crystals are soluble in ammonia and hydrochloric acid, and insoluble in acetic acid, water, alcohol, and ether. They can thus be readily distinguished from certain forms of uric acid, with which they might possibly be confounded at first sight. When heated upon platinum foil they burn with a bluish-green flame without melting.

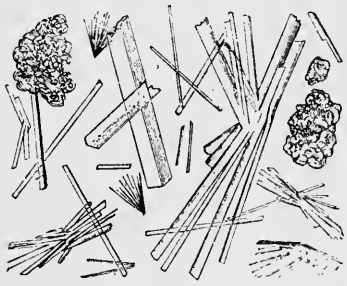


FIG. 169.—Calcium sulphate crystals. (v. Jaksch.)

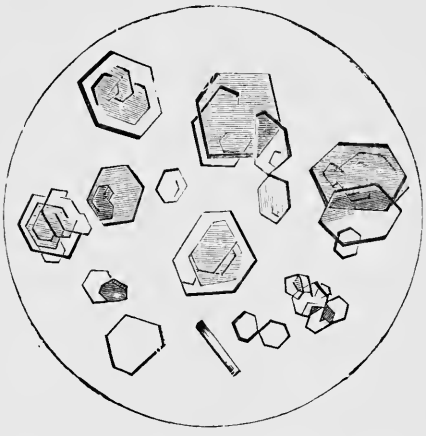


FIG. 170.—Crystals of cystin spontaneously voided with urine. (Roberts.)

Cystin-containing urines may be of normal appearance, but they often present a peculiar greenish-yellow color. The reaction is mostly neutral or alkaline. Upon standing exposed to the air a marked odor of hydrogen sulphide develops, owing to decomposition of the cystin; but at times urines are met with in which a distinct odor of hydrogen sulphide is noticeable, although crystals of cystin are not seen in the sediment. It may then be demonstrated by strongly acidifying the urine with acetic acid or by allowing it to undergo ammoniacal decomposition. In either case cystin crystals will separate out on standing. It should be remembered, however, that not all urines in which hydrogen sulphide is formed contain cystin. (See Hydrothionuria.)

The amount of cystin which may be found in urinary sediments is variable. Sometimes a few crystals only are obtained, while at others from 0.5 to 1 gram may be recovered. As in the case with the other non-organized constituents of sediments, however, the amount deposited does not necessarily indicate the total amount present. Where a quantitative estimation of cystin is to be made, it is best to filter off that which is deposited and to estimate the amount of neutral sulphur in the filtered urine. An increase beyond the normal may be referred to the cystin remaining in solution. (See Neutral Sulphur.)

Clinical interest in connection with cystinuria centres in the frequent association of cystin sediments with cystin gravel or calculi; but the cystinuria may exist for years without giving rise to clinical symptoms.

Very remarkable is the not uncommon occurrence of cystinuria in families. Cases of transient cystinuria likewise occur, and it is hence scarcely admissible to speak of a "cured" cystinuria when the condition disappears under some supposed treatment.

Of the origin of the condition little is known. It has been supposed that the appearance of cystin in the urine is in some manner connected with the formation of certain diamins in the intestinal canal. I have pointed out, however, that in all probability the formation of cystin and diamins takes place in the tissues of the body, and that the appearance of both is the expression of a definite metabolic anomaly rather than of a specific infection. (See Ptomains and Neutral Sulphur.)

Leucin and Tyrosin.—These are never found in urinary sediments under normal conditions. They are seen especially in acute yellow atrophy and in some cases of acute phosphorus poisoning.

Traces of leucin and tyrosin are said to be constantly present also in cases of cirrhosis and carcinoma of the liver, in cholelithiasis, catarrhal jaundice, Weil's disease, nephritis, cystitis, gout, bronchitis, tuberculosis, typhoid fever, hysteria, erysipelas, glucosuria, etc., but in many cases the proof has not been properly furnished that the substance under examination was really tyrosin. In connection with cystinuria the elimination of tyrosin has also been observed, but in two cases which I have examined in this direction I obtained negative results.

Isolation of Leucin and Tyrosin.—As leucin is hardly ever found in the sediment, and tyrosin only when present in large quantities, the urine in every case should first be concentrated upon a water bath and examined on cooling. At times, however, when these substances are present in only very small quantities, this procedure may not lead to the desired end, and in doubtful cases the following method should be employed:

The total amount of urine voided in twenty-four hours is pre-

cipitated with basic lead acetate and filtered, when the filtrate, from which the excess of lead has been removed by means of hydrogen sulphide, is evaporated to as small a volume as possible. Any urea that may be present is removed by shaking with a small amount of absolute alcohol, and the insoluble residue extracted with alcohol containing a little ammonia. This extract is concentrated to a small volume and left to spontaneous crystallization. Leucin and tyrosin separate out and can then be further examined.

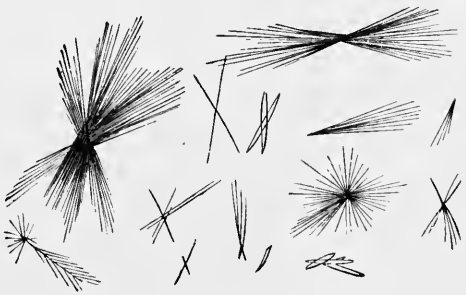


FIG. 171.—Tyrosin crystals. (Charles.)

Ulrich advises to evaporate the urine to dryness and to heat the residue gently while the vessel is covered with a plate of glass or a funnel. The tyrosin is then said to sublime, and is deposited on the cool glass in crystalline form, the crystals giving the characteristic reactions.

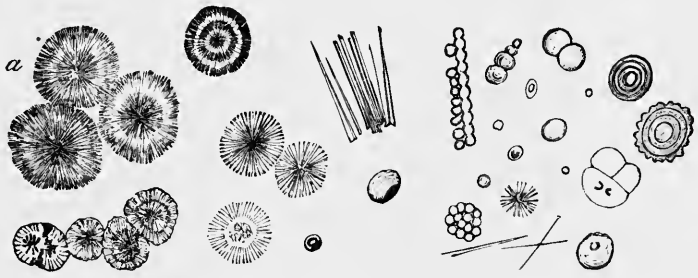


FIG. 172.—Crystals of leucin (different forms). (Crystals of kreatinin-zinc chloride resemble the leucin crystals depicted at *a*.) The crystals figured to the right consist of comparatively impure leucin. (Charles.)

Tyrosin crystallizes in the form of very fine needles (Fig. 171), which are usually grouped in sheaves. They are insoluble in acetic acid, but soluble in ammonia and hydrochloric acid.

Leucin (Fig. 172) occurs in the form of spherules of variable size, which resemble globules of fat, but may be distinguished from these by their insolubility in ether. In the urine they present a more or

less pronounced brownish color, and upon close examination concentric striations as well as very fine radiating lines can at times be made out, which are especially characteristic.

If crystals resembling tyrosin and leucin are found, the following tests should be made:

Separation of the Tyrosin.—The crystals are filtered off, washed with water, and dissolved in ammonia to which a little ammonium carbonate has been added. The solution is allowed to evaporate, when the tyrosin separates out.

Piria's Test.—A bit of the tyrosin is moistened on a watch crystal with a few drops of concentrated sulphuric acid, covered, and set aside for half an hour. It is then diluted with water, heated, and while hot saturated with calcium carbonate and the solution filtered. The filtrate is colorless, but when heated with a few drops of a very dilute neutral solution of ferric chloride it assumes a violet tint.

Hoffmann's Test.—A small amount of tyrosin is dissolved in hot water and treated, while hot, with mercuric nitrate and potassium nitrite. The solution assumes a dark-red color and yields a voluminous red precipitate.

Separation of the Leucin.—After filtering off the tyrosin from the ammoniacal solution (see Separation of Tyrosin, above) the residual fluid is concentrated to the point of crystallization and treated with a little alcohol. This will take up the leucin. The alcoholic extract is allowed to evaporate and the residue examined with Scherer's test.

Scherer's Test.—When leucin is treated upon platinum foil with nitric acid, a colorless residue is obtained which, upon the application of heat and the addition of a few drops of a solution of sodium hydrate forms a droplet of an oily fluid which does not adhere to the platinum.

Hofmeister's Test.—A small amount of leucin dissolved in water causes a deposit of metallic mercury when heated with mercurous nitrate.

Xanthin Crystals.—Xanthin crystals (Fig. 173) are very rarely observed in urinary sediments, and, so far as I have been able to ascertain, the case observed by Bence Jones is the only one on record. Care should be had not to confound colorless crystals of uric acid with xanthin, which is readily soluble in ammonia.

Xanthin sediments may be recognized by means of the following test: A small amount of the material is treated with a few drops of concentrated nitric acid on a porcelain plate, and evaporated to dryness. In the presence of xanthin a yellow residue is obtained, which turns a violet red upon the addition of a few drops of sodium hydrate solution and the application of heat (Strecker's test). The reaction is common to all the xanthins and should not be confused with the murexide test.

Clinically, xanthin sediments are of interest only in so far as this substance may give rise to the formation of calculi.

Soaps of Lime and Magnesia.—Von Jaksch has pointed out that in various diseases crystals may be found which "closely" resemble tyrosin in appearance, and pictures such crystals (Fig. 174), which from their behavior toward reagents he is inclined to regard as calcium and magnesium salts of certain higher fatty acids.

Should doubt arise, the question may be readily decided by a chemical examination. (See Tests for Tyrosin and Fatty Acids.)

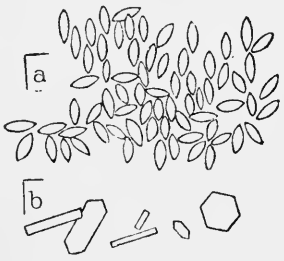


Fig. 173.—*a*, crystals of xanthin. (Salkowski.) *b*, crystals of cystin. (Robin.)

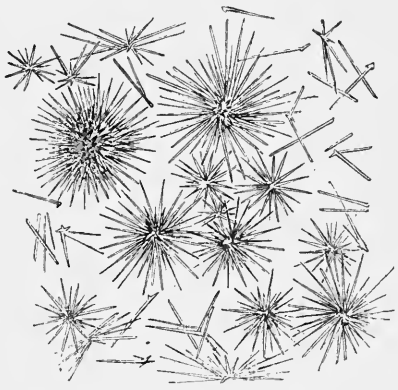


Fig. 174.—Lime and magnesium soaps. (v. Jaksch.)

Bilirubin (Hematoidin) Crystals.—Bilirubin (hematoidin) crystals in the form of yellow or ruby-red rhombic plates or needles, as well as amorphous granules, have been seen in the urine in rare cases. They are easily soluble in alkalies and chloroform, but not in ether. When treated upon a slide with a drop of nitric acid a green ring will be seen to form around them (Gmelin's reaction). Such crystals have been found either free or embedded within cells or tube casts, in cases of scarlatinal nephritis, the nephritis of pregnancy, in granular atrophy, amyloid degeneration of the kidneys, in icteric urines and in carcinoma of the bladder, of which latter condition they have been regarded by some as pathognomonic.

Fat.—When small, strongly refractive globules of fat, which may be readily recognized by their solubility in ether, are observed either floating on the urine or held in suspension, it is necessary to ascertain first of all whether such fat may not be present accidentally, owing to the use of a bottle or vessel not absolutely clean, or previous catheterization, etc. The diagnosis *lipuria* should only be made when all possible precautions have been taken to exclude the *accidental* presence of this substance. True lipuria—*i. e.*, an elimination of fat usually in the form of droplets floating on the urine—

has been noted in various cachectic conditions, in cases of heart disease, affections of the pancreas and liver, in gangrene and pyemia, in diseases of the bones, especially following fractures, in diseases of the joints, in diabetes, and notably in chyluria. Fat has also been observed in the urine following the ingestion of large amounts of cod-liver oil and inunctions with fats and oils.

In fatty degeneration of the kidneys (nephritis, phosphorus poisoning, etc.), droplets of fat may be seen in the epithelial cells and tube casts. This, however, does not constitute lipuria. The nature of the droplets may be recognized by their solubility in ether, benzol, chloroform, carbon disulphide, xylol, etc., and by the fact that they are colored black when treated with a 0.5 to 1 per cent. solution of osmic acid, and red when a drop of tincture of alcanna is added to the specimen. A convenient method of demonstrating the presence of fat is also the following: A few cubic centimeters of the urine are mixed with an equal volume of 96 per cent. alcohol and a concentrated solution of Sudan III in 96 per cent. alcohol. The sediment which collects is then examined under the microscope; the excess of stain is removed by allowing a few drops of 60 or 70 per cent. alcohol to run under the cover-slip and removing it with filter paper placed at the edge of the preparation. The fat droplets are thus colored a vermilion red. Free fat can, of course, be demonstrated in the same manner. (See also Lipuria.)

Sediments Occurring in Alkaline Urines.—Basic Phosphate of Calcium and Magnesium.—The most common sediments observed in alkaline urines consist of amorphous phosphates of calcium and magnesium. They are usually as abundant as the urate sediments which have been described, but may be distinguished from these by the fact that they do not dissolve upon the application of heat, but disappear upon the addition of acetic acid, and are never colored. In this manner it is also easy to distinguish such a sediment from one due to pus, with which it might possibly be confounded at first sight. Upon microscopic examination a droplet of the sediment will be seen to contain innumerable transparent granules scattered over the entire field, and closely resembling those of urate of sodium.

Phosphatic sediments are observed, as mentioned elsewhere, whenever the reaction of the urine is alkaline, whether this be owing to the presence of fixed alkali or to ammoniacal fermentation. They also result if a faintly acid, faintly alkaline, or amphoteric urine is boiled.

Neutral Calcium Phosphate.—These crystals may be found in alkaline, amphoteric, and feebly acid urines, but are not very common. They are at times of large size, but more commonly acicular, occurring either singly or united in a star-like manner (Fig. 175). They are colorless, readily soluble in acetic acid, and insoluble in warm water, so that they can be easily distinguished from uric acid.

Basic Magnesium Phosphate.—Crystals of this salt occurring in the form of large, highly refractive plates (Fig. 176) are at times seen in alkaline, neutral, or faintly acid and highly concentrated urines. They are readily recognized by treating a drop of the sediment upon a slide with a drop of ammonium carbonate solution (1 to 4), when the crystals become opaque and their edges assume an eroded aspect. In acetic acid they dissolve with ease, and may then be reprecipitated by means of sodium carbonate. They are uncommon.

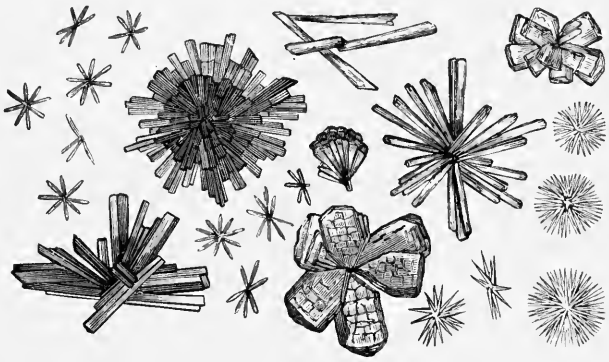


Fig. 175.—Crystalline phosphates. (Finlayson.)

Ammoniomagnesium Phosphate.—This salt, usually spoken of as triple phosphate, crystallizes in large prismatic crystals of the rhombic system; it is most abundantly observed in alkaline urines, but may also occur in feebly acid specimens. Of the various forms which may occur, that resembling the lid of a German coffin is the most characteristic (Fig. 177, *a*). At times these crystals attain a large size; very small specimens, however, also occur which may be mistaken for oxalate of calcium, but from these they are distinguished by the ease with which they dissolve in acetic acid.

Fig. 176.—Basic magnesium phosphate crystals. (v. Jaksch.)

Here, as elsewhere, it should be remembered that no conclusions as to the amount actually eliminated can be drawn from a microscopic examination, and the diagnosis "phosphaturia" should be based only upon the results of a quantitative analysis.

The continued elimination of a turbid urine, the turbidity of which is referable to phosphates, is notably observed in certain neurasthenic individuals with a predominance of cerebral symptoms. Very curiously the phosphaturia is not influenced by diet.

Fig. 177.—Various forms of triple phosphates. (Finlayson.)

Calcium Carbonate.—Calcium carbonate frequently occurs in alkaline urines, and appears under the microscope in the form of minute granules, occurring singly or arranged in masses; dumb-bell forms are also seen (Fig. 178). They may be recognized by the fact that they readily dissolve in acetic acid, with the evolution of gas.

Fig. 178.—Calcium carbonate crystals.

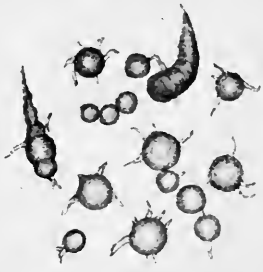


Fig. 179.—Ammonium urate crystals.

Ammonium Urate.—Ammonium urate is observed only in urines which are undergoing ammoniacal decomposition. Its presence should always call for a careful investigation in order to ascertain whether this has taken place after the urine has been voided or before. (See Reaction.)

The salt occurs in the form of brownish, spherical bodies of variable size, which are sometimes composed of delicate needles, while at

others they are amporphous, but may be beset with prismatic sicules (thornapple forms). They are not easily mistaken for any other substance which may be present in urinary sediments (Fig. 179). Ammonium urate is characterized, moreover, by its solubility in acetic and hydrochloric acids, and by the subsequent separation of rhombic crystals of uric acid.

Indigo.—Indigo in the form of delicate blue needles, arranged in a stellate manner or in plates, visible only with the microscope, is rarely seen. In an amorphous condition, however, it may be met with in almost every decomposed urine, occurring in the form of small granules and sometimes staining the morphological elements that may be present a distinct blue. Sediments presenting a bluish-black color were noted in the time of Hippocrates already, and have been described since by numerous observers, but the nature of the coloring matter has only been determined within the last fifty years. Clinically, the occurrence of indigo in the urine is of interest, as renal calculi have been observed which consisted almost entirely of this substance. But little is known of the causes which give rise to its appearance in the urine, but there can be no doubt that its occurrence is referable to the action of certain microörganisms upon urinary indican (which see).

ORGANIZED CONSTITUENTS OF URINARY SEDIMENTS

Epithelial Cells (Fig. 180).—Bearing in mind the fact that desquamative processes are constantly going on in the epithelial lining of the various cavities and channels of the body, one should expect to find in every urine representatives of the different forms of epithelium from the Malpighian tufts down to the meatus urinarius. To a certain extent this actually happens, and cells apparently derived from the meatus, the urethra, bladder, ureters, and pelvis of the kidneys may be met with in almost every specimen, although it is often difficult to tell the origin of the individual cells. Bizzozero even claims that it is impossible to distinguish between the cells of the bladder and those of the meatus and renal pelvis, while as a class they may be differentiated in most cases from the cells of the urethra, the ureters, the prepuce of the male, and the vulva and vagina of the female. Cells from the uriniferous tubules are not seen in normal urines.

The number of epithelial cells occurring in urinary sediments under physiological conditions is small, and the presence of large numbers may hence always be regarded as abnormal. Their appearance is influenced by the reaction of the urine, an alkaline or neutral urine causing them to swell and to appear larger and rounder than in acid urines. As has been mentioned, the cellular type is practically the same, moreover, in the bladder, ureters, and pelvis of the kidneys.

As has already been stated, it may be very difficult to determine the origin of single epithelial cells, or even of groups of cells, by examining these *per se*. But not infrequently other findings may lead to their proper classification and interpretation.

Generally speaking, three forms of epithelial cells may be found in urinary sediments, viz.:

1. Round cells.
2. Conical and caudate cells.
3. Flat cells.

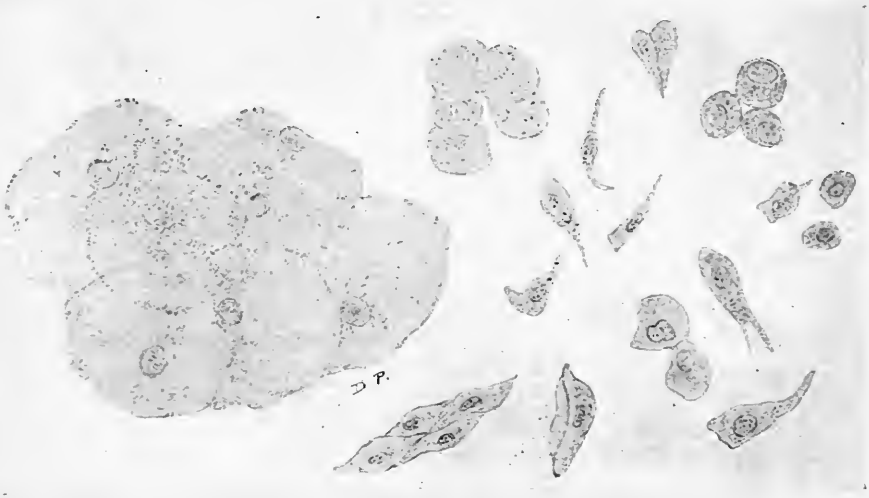


Fig. 180.—Urinary epithelium.

Round Cells.—These may be derived from the uriniferous tubules or the deeper layers of the mucous membrane of the pelvis of the kidneys. They are somewhat larger than pus corpuscles and may be distinguished from these by the presence of a large, well-defined nucleus, which is readily visible as such, while in pus cells it becomes distinct only upon the addition of acetic acid, and is, moreover, multiple. Whenever such cells are found adhering to urinary casts, it is clear that they represent the glandular elements proper of the kidneys. As similar cells are found in the male urethra, confusion may arise. Should albumin be present, the cells are probably of renal origin. The presence of such cells in large numbers together with pus, in the absence of tube casts and albumin beyond traces, will usually indicate the existence of a simple pyelitis, particularly if the cells are found joined in a shingle-like manner. Should the pyelitis be associated with a nephritis, tube casts and albumin in larger amounts will at the same time be present. In such cases it

may be impossible to determine the origin of the cells, excepting of such that may adhere to casts.

In simple circulatory disturbances affecting the renal parenchyma no special abnormalities can be discovered in the structure of the cells, while in fatty degeneration of the kidneys they will be seen to contain fatty particles in greater or less abundance. At other times they are markedly granular and occur in fragments.

Conical and Caudate Cells.—These cells are mostly derived from the superficial layers of the pelvis of the kidneys, and are hence seen in large numbers in cases of pyelitis. Similar cells, however, are also found in the neck of the bladder.

Flat Cells.—Flat cells may come from the ureters, the bladder, or the genitalia. Large polygonal cells provided with single distinct nuclei and a more or less markedly granular protoplasmic zone about the nucleus are usually derived from the external genitals. Many such cells are more or less broken down and distorted. The surface cells from the bladder and ureters are less apt to show evidence of injury or degeneration, and are, on the whole, smaller. Surface epithelial cells from the vagina are mostly fusiform in shape and very commonly show an irregular, warped outline. Often they are seen in large plaques. Other more or less rounded forms are derived from the deeper layers of the mucosa. Irregular or conical cells, often provided with one or more protoplasmic processes, likewise come from the lower layer of the mucosa of the bladder and ureters.

In alkaline urines undergoing bacterial decomposition it is common to meet with large surface epithelial cells from the external genitals which are literally one mass of bacteria.

Leukocytes.—Leukocytes are encountered in only very small numbers in normal urines. A marked increase should, hence, always be regarded as indicating the existence of disease somewhere in the urinary tract, excepting in females, where their presence may be owing to an admixture of leucorrheal discharge. In that case the source of the pus will generally be recognized by the simultaneous occurrence of pavement epithelial cells of the vaginal type in correspondingly large numbers. In doubtful cases the urine should always be obtained with the catheter, care being taken to thoroughly cleanse the vulva before the introduction of the instrument.

Occasionally the pus is derived from a neighboring abscess that has opened into the urinary passages.

The amount of pus which may be found in urines is most variable. On the one hand, deposits several centimeters in height are not uncommon, and closely resemble deposits of phosphates, for which they are, indeed, frequently mistaken; on the other hand, it may only be possible to discover the presence of pus by means of the microscope, which should be employed in every case.

The appearance of the pus corpuscles varies in different cases.

In acid urines their form is usually well preserved, and in feebly alkaline and neutral specimens it may even be possible to observe ameboid movements when the slide is carefully warmed. In alkaline urines, however, they usually swell up and become opaque, so that it is impossible to discern a nucleus unless they are treated with acetic acid. At other times, and particularly when pus has remained long in the body, it may be almost impossible to make out a nucleus, and in extreme instances nothing but a mass of granular and fatty detritus is left.

While with a certain amount of experience it is hardly likely that a sediment of pus will be mistaken for anything else, it should be remembered that if pus is exposed to the action of ammonia or an ammonium salt the pus corpuscles become disintegrated. In such cases, as in old, neglected instances of cystitis, in which ammoniacal decomposition of the urine has taken place in the bladder, a deposit may be obtained which microscopically resembles mucus, and in which pus corpuscles may not even be demonstrable with the microscope. The sediment escapes as a gelatinous, slippery mass when the urine is poured from one vessel into another. Recourse must then be had to certain chemical tests, as a pyuria might otherwise be overlooked. To this end the following procedure, suggested by Vitali, may be employed:

The urine, after having been acidified with acetic acid, is filtered, and the contents of the filter treated with a few drops of tincture of guaiacum which has been kept in the dark, when in the presence of pus the filter paper is colored a deep blue.

A solution of iodopotassic iodide may be employed in less extreme instances. A drop of this solution is added to a drop of the sediment upon a slide, when the pus corpuscles, owing to the presence of glycogen, are colored a dark mahogany brown, while epithelial cells, with certain forms of which they might possibly be mistaken, assume a light-yellow color.

Donné's Pus Test.—This test is based upon the fact that the transformation of pus into a gelatinous, mucus-like mass, observed in cases of cystitis, owing to the action of ammonium carbonate, may also be artificially produced by the addition of a small piece of caustic soda and stirring, when in the presence of pus in small amounts the liquid becomes mucilaginous and ropy, while a gelatinous mass is obtained if it is abundant.

Müller's Modification of Donné's Test.—5 to 10 c.c. of urine are treated drop by drop with official sodium hydrate solution, shaking thoroughly after the addition of each drop. If then the tube is observed, it will be noted that the bubbles of air can rise only very slowly through the viscid fluid or in the presence of fair amounts of pus may remain stationary altogether. A positive reaction is still obtained from 1200 pus cells to the c.mm.

Clinical Significance of Leukocytes in Urine.—From a clinical point of view it is important to establish the source of the pus in every case of *pyuria*. This may at times be difficult, but the following data will be found of value in a differential diagnosis:

1. In disease affecting the renal parenchyma the amount of pus, as a rule, is small, except where a large abscess located in the kidney structure proper has burst into the pelvis of the kidney.

In uncomplicated cases it is a comparatively easy matter to recognize the renal origin of the pus, as other constituents, such as renal epithelial cells, and tube casts, are usually present at the same time, and, as was noted in the case of renal epithelial cells, leukocytes are frequently found adhering to the tube casts, and at times apparently compose these entirely, when they are spoken of as *pus casts*. (See Casts.) In nephritis, according to Bizzozero, the number of pus corpuscles stands in a direct relation to the intensity and acute character of the morbid process, the greatest number being found in cases of acute nephritis, while in chronic forms their number is usually insignificant. Whenever in the course of a chronic nephritis large numbers of pus corpuscles appear they may be regarded as indicating either an acute exacerbation of the disease or a complicating inflammation of some portion of the urinary tract. In such cases errors may be guarded against by observing the number and character of the epithelial cells present at the same time, when it will often be found that what at first sight appears as an acute exacerbation of a chronic process, judging from the number of pus corpuscles, is in reality a secondary pyelitis, ureteritis, or cystitis.

In cases of simple renal hyperemia pus corpuscles never occur in notable numbers.

2. In pyelitis the amount of pus may vary considerably, and at times even perfectly clear urine may be voided. This is probably owing to the fact that the ureter of the affected side, if the disease is unilateral, becomes obstructed temporarily, when suddenly large quantities may appear again. In other cases in which the morbid process is confined to one of the calices this may become shut off temporarily, so that a clear urine results. The diagnosis of pyelitis is often difficult, and should be based not only upon the condition of the urine, but upon clinical symptoms as well. Very significant is the fact that the urine in pyelitis is usually acid. A careful examination of the epithelial elements may also be of value, and should never be neglected. Bacteria in large numbers are generally present.

In renal tuberculosis pus appears very early, but the amount may be extremely variable. Sometimes only a few leukocytes are seen, while at other times it may amount to one-fourth and even one-half of the urine by volume. As a rule, the pyuria is constant, but cases are seen where for months and even years the urine may be almost clear and the condition is much improved. It should be remem-

bered, however, that the passage of apparently normal urine may merely indicate that the other ureter is blocked.

When pyelitis is associated with nephritis it may at times be almost impossible to determine the origin of the pus; but if the rule set forth above is remembered, that in chronic nephritis the number of leukocytes is small, it is not likely that a pyelitis will be overlooked, particularly if the clinical symptoms are taken into consideration.

Matters may become still more complicated when a cystitis is accompanied by a pyelitis or a pyelonephritis. Catheterization of the ureters should then be resorted to. Fischl regards the presence of cylindrical masses composed of pus corpuscles, formed in all probability in the papillary ducts, as highly characteristic of pyelitis.

3. A pyuria referable to ureteritis can hardly be diagnosticated from the appearance of the urine, and in suspected cases catheterization of the ureters should be resorted to, which will probably throw light upon the question.

4. In mild cases of cystitis pus may be altogether absent, while in the more severe forms its presence is constant. In cystitis the largest amounts referable to disease of the urinary organs are observed, and are exceeded only in those rare conditions in which a neighboring abscess has opened into the urinary passages.

As the urine in cystitis is commonly alkaline, and always so in the more severe forms, the alkalinity being due to ammoniacal fermentation, it may happen, owing to the disintegrating action of the ammonium carbonate upon the pus corpuscles, that these may not be demonstrable with the microscope, and that a gelatinous mucoid sediment appears instead, which escapes from the vessel *en masse* when the urine is poured out. The chemical tests for pus, described above, must then be employed.

5. In urethritis pus may be present in the urine in considerable amount. The source of the pus is recognized by the fact that a drop may be manually expressed from the urethra, particularly in the morning upon awaking. Mucoid gonorrheal threads—the "Tripperfäden" of the Germans—which are largely composed of pus corpuscles, will almost always be detected in the urine in such cases. In order to distinguish between a simple urethritis and a urethritis complicated with cystitis, the urine should be obtained in two portions and allowed to settle. In simple urethritis affecting the anterior portion of the urethra the first specimen is cloudy, while the second one is clear. If the urethritis, however, has extended to the neck of the bladder, in the absence of cystitis, the first portion will, of course, be cloudy, while the second may present a variable appearance, being clear at times and cloudy at others. This phenomenon is explained by the fact that a portion of the pus contained in the posterior portion of the urethra has found its way into the bladder. A cystitis may, however, be excluded by the acid reaction of the second speci-

men, and the fact that the latter is never so cloudy as the first. In cases of urethritis complicated with a purulent cystitis the second portion of the urine contains at least as much pus as the first, and usually more, owing to the fact that the pus (which is heavier than the urine) falls to the floor of the bladder, in which case also the last drop passed will often be found to be pure pus. The reaction of the urine, moreover, will then be generally alkaline.

6. A sudden elimination of large quantities of pus with a urine which up to that time has presented a normal or nearly normal appearance may almost always be referred to rupture of an abscess into the urinary passages. Exceptions to this rule have been noted in rare instances in which large amounts of pus suddenly appeared, the origin of which could not be demonstrated upon postmortem investigation. Whether such a phenomenon, as v. Jaksch suggests, is dependent upon "unusual conditions favoring diapedesis" remains an open question.

Enumeration of the Pus Corpuscles in the Urine.—In order to determine the relation existing between the degree of pyuria and albuminuria, as well as to watch the progress of an individual case, an enumeration of the number of pus corpuscles is at times necessary. To this end a specimen of the urine is thoroughly shaken and the number of corpuscles contained in one cubic millimeter ascertained with the aid of the hemocytometer (Simon's ruling). Dilution with a 3 per cent. solution of common salt is necessary if a preliminary examination has shown the presence of more than 40,000 corpuscles per cubic millimeter. A dilution of five times is usually sufficient.

Some of the results which have thus been obtained are extremely interesting. In cases of mild cystitis 5000 pus corpuscles are found on an average in the cubic millimeter; in cases of moderate severity from 10,000 to 20,000; while in severe cases 50,000 and even more may be seen. In one case of cystitis complicating carcinoma of the bladder, Hottinger obtained 152,000 per c.mm. In the presence of less than 50,000 a mere trace of albumin is found, and with 80,000 to 100,000 only 1 pro mille is referable to this source.

Red Blood Corpuscles.—The presence of red blood corpuscles in the urine, constituting the condition usually spoken of as *hematuria*, is observed only in pathological conditions, and is, in contradistinction to hemoglobinuria (which see), a relatively common occurrence.

Urine containing blood corpuscles in notable numbers presents a color which may vary from a bright red to a dark brown verging upon black. Upon standing, a sediment of a corresponding color is obtained in which distinct coagula of variable size are at times seen.

If the urine should contain only a small number of red corpuscles, however, no deviation from its normal appearance will be noted, and the diagnosis of hematuria can then only be made with the micro-

scope, which should be employed in every case. The appearance of the red corpuscles varies greatly, being influenced especially by the length of time during which they have remained in the urine. In cases of hematuria of urethral or vesical origin it will be found that they have mostly retained their normal appearance fairly well, or have become crenated, when they may be recognized without difficulty. In cases, on the other hand, in which the corpuscles have remained in the urine for a longer time, as in hematuria of renal origin, the inexperienced will frequently be puzzled by the presence of bodies of the size of red corpuscles, or somewhat smaller, which are entirely devoid of coloring matter, and appear as faint, transparent rings, often presenting a double contour, and in which no nucleus can be discovered. These formations are red blood corpuscles from which the hemoglobin has been dissolved. They are spoken of as *blood shadows*. Chemical tests are rarely necessary, but may be employed if doubt should arise.

Clinical Significance of Red Cells in Urine.—Clinically it is, of course, all-important to determine the source of the blood. This may at times be accomplished without much difficulty by a urinary examination, but at other times it may almost be impossible, when the clinical symptoms and physical signs must be taken into consideration.

1. Hematuria of urethral origin, due to urethritis, prostatitis, or traumatism incident to catheterization, for example, is a common event, and readily diagnosticated, as in such cases blood either escapes of itself from the urethra or it may be squeezed out manually. The last portion of the urine voided, moreover, will always be found free from blood, unless it is referable to disease of the neck of the bladder, when the blood appears only toward the end of micturition, or at least more markedly then than in the beginning.

2. The diagnosis of vesical hematuria is not always easily made. It should be remembered, however, that the blood corpuscles here present a normal appearance, as has been mentioned, unless ammoniacal decomposition is occurring in the bladder, in which case blood shadows are seen in large numbers. The blood, moreover, is less intimately mixed with the urine than in cases of renal hematuria, so that the corpuscles rapidly settle after the urine has been passed. Blood clots of an irregular form and considerable dimensions can only be of vesical origin. A careful examination for the presence of any other morphological constituents which may be observed in urinary sediments, when considered in conjunction with the clinical symptoms, will usually lead to a correct diagnosis so far as the seat of the hemorrhage is concerned. Hematuria of vesical origin may be due to numerous causes, among which may be mentioned hemorrhagic cystitis, stone, tubercular ulceration, malignant growths, papilloma, traumatism, the presence of parasites, and, more rarely,

rupture of varicose veins in the bladder. In determining the cause of the hemorrhage in a given case, more reliance should be placed upon the clinical history and a direct examination of the bladder than upon the urinary examination.

3. In hematuria of ureteral origin characteristic blood coagula, corresponding in diameter and form to the ureters, are occasionally seen. Their presence, however, does not necessarily indicate that the blood has come from the ureters; more frequently the hemorrhage will be found to be due to disease of the pelvis of the kidney.

4. The diagnosis of hemorrhage into the pelvis of the kidney must be based upon the clinical symptoms taken in conjunction with the results of a urinary examination. In nephrolithiasis only a small number of red corpuscles is usually found, which is important from the standpoint of differential diagnosis. In renal tuberculosis, hematuria is one of the most important symptoms, and not infrequently the first which attracts the attention of the patient. The amount is variable; sometimes the bleeding is microscopic, while in others almost pure blood is passed. It is usually intermittent, the periods of bleeding lasting from one hour to several weeks, the average being three days. Late in the disease it is usually less in amount, but apt to be almost continuous. As a rule, the urine and blood are intimately mixed. Clotting, however, may occur in the bladder and the pelvis of the kidney.

5. Hematuria of purely renal origin is of common occurrence, and may be due to numerous causes. In simple hyperemic conditions of the organs and in hemorrhagic nephritis the passage of smoky looking urine containing blood corpuscles, usually in large numbers, is thus a fairly constant symptom. In chronic nephritis the number of the red corpuscles may be taken to indicate the intensity of the morbid process. Hematuria may also be due to renal abscess, renal tuberculosis, malignant growths, stone, and, in rare instances, to aneurysm and embolism of the renal artery, thrombosis of the renal vein, papilloma of the pelvis, etc. In the malignant forms of the acute infectious diseases, such as smallpox, yellow fever, malaria, etc., in scurvy, hemophilia, and purpura, in leukemia, filariasis, and distomiasis, renal hematuria is common. It is also observed in cases of poisoning with turpentine, carbolic acid, cantharides, and has recently also been observed in several convalescents from typhoid fever while under treatment with urotropin; the hematuria ceased with the discontinuance of the drug.

6. An idiopathic form of hematuria has also been described, in which hemorrhage from the kidneys occurs without apparent cause. This is relatively common. Senator speaks of it as renal hemophilia. It has repeatedly led to errors in diagnosis and more particularly in connection with renal tuberculosis, as it also is usually unilateral.

The amount of blood is very variable, sometimes only microscopic, at others excessive. I have seen three cases of this kind in which no lesion existed which could be made responsible for the hemorrhage. In all three the attacks of hematuria were associated with anachlorhydria, while normal values were found between the attacks. Two of the patients were males, and undoubtedly neurasthenics. The third was an hysterical, chlorotic female, in whom hematemesis, pulmonary hemorrhages, and melena were also at times observed.

Hematuria of renal origin is usually recognized without much difficulty, as in such cases tube casts bearing red blood corpuscles, and at times apparently consisting of these altogether, as well as numbers of renal epithelial cells, will usually be found upon examination. The blood, moreover, is intimately mixed with the urine, and the individual corpuscles have mostly lost their hemoglobin and appear as mere shadows. The clinical history should, of course, always be taken into consideration, especially in determining the primary cause of the hemorrhage.

Urine containing red blood corpuscles is always albuminous, so that it may sometimes be difficult to decide in a given case whether the albumin found is due solely to the presence of blood or whether the hematuria is complicated with an albuminuria *per se*. Frequently it is possible to arrive at some conclusion by comparing the amount of albumin with the number of the red corpuscles, the presence of a large amount of the former in the presence of only a small number of the latter, indicating that the albumin is not altogether due to the blood. At other times it is impossible to gain information in this manner, when the only expedient left is to determine the quantity of albumin and of iron separately, and to ascertain whether the amount of iron found is sufficient to combine with that of the albumin. As a rule, however, the presence of serum albumin, aside from that contained in the blood of the urine, may be inferred whenever tube casts are present, although the amount can only be estimated approximately in this manner.

Tube Casts.—In various pathological conditions, and it is claimed even in health, curious formations are seen in the urine, which represent moulds of different portions of the uriniferous tubules. To these the term *tube casts* or *urinary cylinders* has been applied. The term "tube casts," however, is not altogether appropriate, as it is applicable to only one great division of such formations—*i. e.*, to those consisting of a uniform, transparent, gelatinous matrix, to which other elements, such as epithelial cells, red blood corpuscles, leukocytes, and salts in a crystalline or amorphous form, may accidentally have become attached—*the tube casts proper*.

From these the so-called "pseudocasts" must be differentiated, a pseudocast being characterized essentially by the absence of a uniform matrix. Closely related apparently to the true casts are the

so-called cylindroids—*i. e.*, band-like formations which resemble the former in appearance, and like these may carry various morphological elements. It is thus necessary to distinguish between true casts, pseudocasts, and cylindroids. Of these, the true casts are the most important. They may be divided into hyaline and waxy casts, the two forms being readily differentiated by the fact that the former readily dissolve in acetic acid, while the waxy casts are either not affected at all by this reagent, or, if so, at least not so rapidly. The latter, moreover, are more strongly refractive, to which property their waxy appearance is due; their color is slightly yellow or yellowish gray, while the hyaline casts are colorless and usually very pale and transparent.

Mode of Examination.—Unless a urine can be examined within a few hours after being voided, it is well to add a small amount of chloroform, so as to guard against bacterial decomposition. The use of conical glasses is unsatisfactory, and I find it more convenient to keep the urine in well-stoppered bottles. Preserved with chloroform it will keep almost indefinitely. Where a centrifugal machine is available the specimen can, of course, be examined at once. As soon as a sufficient amount of sediment has been obtained, a few drops are spread on a slide and examined, *uncovered, with a low power. It is essential, however, to make use of the flat mirror and to avoid a bright light. If this is borne in mind, no difficulty whatever will be found in demonstrating even the most hyaline specimens, though they may be present in very small numbers.* In many textbooks on urinary analysis the writers speak of the difficulty attending the search for hyaline casts, and the advice is frequently given to color the preparations with a drop of a dilute aqueous solution of iodopotassic iodide, or of some other staining reagent, such as gentian violet, picrocarmin, methylene blue, or osmic acid. This is unnecessary if the directions just given are strictly followed. If a *bright* light is used, however, I am willing to admit that even the most experienced examiner may be unsuccessful in his search.

For the preservation of mounted specimens the following method, devised by Krönig, may be employed, though I personally prefer to keep the urine itself and to mount a fresh specimen when necessary. A drop of the sediment, best obtained by centrifugation, is spread on a cover-glass and allowed to dry in the air. It is then placed in a 10 per cent. solution of formalin for ten minutes, rinsed in water and stained for about ten minutes in a concentrated solution of Sudan III in 70 per cent. alcohol. The excess of stain is removed by immersion for one-half to one minute in 70 per cent. alcohol, when the specimen is counterstained with Ehrlich's hematoxylin, rinsed in water, and mounted in glycerin. Evaporation is guarded against by ringing the specimen with asphaltum. The tube casts are thus stained a more or less pronounced blue, the nuclei of the

leukocytes dark blue, and any fatty granules or needles of fatty acids that may be present a bright red.

I have obtained very satisfactory results by pouring a small amount of a 1 per cent. aqueous solution of eosin into one of the tubes of the urinary centrifuge, filling up with urine and then centrifugating. The supernatant fluid is poured off and the sediment mixed with Farrant's solution; the specimens are finally ringed with asphaltum and keep for a long time. The hyaline casts appear a delicate rose, while the fatty casts are a bright vermilion and the brown, granular casts a reddish brown. Adhering granules or cells are colored a bright red.

Liebmann recommends a mixture of 2 grams of methylene blue dissolved in 100 c.c. of a 10 per cent. solution of formalin. The urine is first centrifugated, the supernatant fluid is poured off, when a few drops of the reagent are poured on the sediment, and left a few minutes. The tube is filled with water, left for a while for the salts to dissolve, then centrifugated again, when the formed elements are ready for microscopic examination.

True Casts.—*Hyaline Casts* (Plate XXV).—Upon careful examination it will be seen that with rare exceptions the matrix of hyaline casts is not *altogether* homogeneous, as small granules may almost be detected embedded in or adhering to the matrix. As these granules occur in greater or less numbers, hyaline casts are spoken of as being finely granular (Plate XXV), coarsely granular, finely dotted, etc. Should true morphological elements be detected, the casts are termed blood casts, epithelial casts, or pus casts (Fig. 181).

The nature of these various forms can probably always be made out without much difficulty, and even in those cases in which the hyaline matrix is apparently concealed beneath cellular elements it will usually be possible, upon closer observation, to detect a fine boundary line at some portion of the structure. Not infrequently also the end of the cast will be seen to be more or less distinctly hyaline. In others, again, a hyaline zone may be observed along the sides of a central organized thread, so to speak, this being frequently seen in specimens which are very broad and long. Should doubt arise, however, a drop of acetic acid is added to a drop of the sediment on the slide; the acid dissolves the hyaline matrix, the organized constituents are set free, and the differential diagnosis between a pseudocast and a compound hyaline cast is thus readily established.

The length of hyaline casts varies greatly. It may scarcely exceed the breadth, on the one hand, while, on the other, although rarely, the casts may traverse the entire microscopic field. In breadth they vary between 0.01 and 0.05 mm. As a rule, the breadth of a cast is uniform throughout its entire length, but specimens are not infrequently observed in which one end tapers considerably and

PLATE XXV

Casts.

a, *a*, waxy casts; *b*, same, stained with eosin; *c*, *c*, *c*, hyaline casts; *d*, same, stained with eosin; *e*, *e*, *e*, brown granular casts; *f*, *f*, coarsely granular casts; *g*, epithelial cast; *h*, blood cast, stained with eosin. (Low-power picture, Leitz 3.)

presents a spirally twisted appearance. This may be so marked that the entire cast appears transversely striated. It is generally

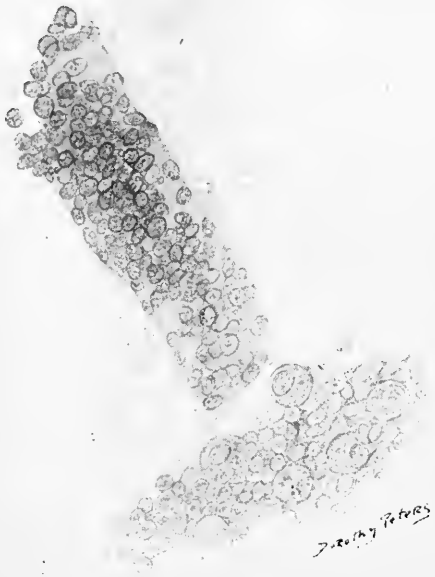


FIG. 181.—Pus and epithelial casts.

supposed that this results from the adhesion of one end of the cast to the walls of a tubule, the lumen of which it does not fill, so that

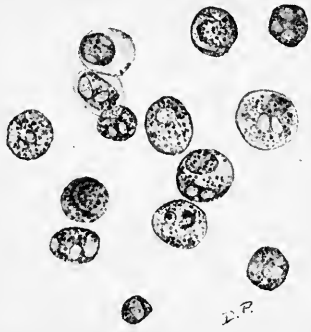


FIG. 182.—Pus cells from a urinary sediment.

the free end becomes twisted in the downward course. A dichotomous branching of one end is also at times seen in very broad hyaline specimens.

Fat globules are frequently found upon hyaline casts and are probably derived from degenerated epithelial cells. When present in large numbers such casts are termed fatty casts. The globules are soluble in ether and are colored red by Sudan III. (See Tests for Fat.)

Granules of melanin, indigo, and altered blood pigment may also at times be observed in casts.

Regarding the mode of formation of the hyaline casts, it is now thought that the matrix is essentially an inflammatory exudate, formed through the activity of the morbidly altered epithelial cells, and subsequently coagulated in the tubules.

Brown Granular Casts.—These should not be confounded with the granular hyaline variety. They show no evidence of a hyaline matrix and on staining with eosin they are colored a deep brownish red (Plate XXV). Unstained they appear brown. They are unquestionably composed of epithelial cells which have undergone degeneration, the residual material being then packed together in cast form. They are quite brittle and often not longer than they are broad. The true nature of these small masses can be made out by staining with eosin, when it will be seen that they stain exactly like the larger pieces that have not yet broken down.

Waxy Casts.—The waxy casts may be divided into two groups—true waxy casts and amyloid casts; but as the latter are not necessarily indicative of the existence of amyloid degeneration of the kidneys, such a classification is of only theoretical interest. They are readily distinguished from the hyaline casts by the characteristics mentioned above—*i. e.*, their higher degree of refraction, their yellow or yellowish-gray color, and the fact that they are either not attacked at all by acetic acid or only very gradually. Their appearance suggests a much more solid object than the hyaline casts. As a rule, only small fragments are found, but in some instances very long casts are seen, and occasionally I have found such long casts which were branching. Some of these casts at times present a peculiar knotty appearance. With eosin the waxy casts are colored a bright vermilion, while hyaline casts show only a pink color. Waxy casts may also contain cellular elements, crystals and amorphous mineral matter; but, as a rule, such compound casts are not so commonly observed as are those of the hyaline variety.

As has been stated, some waxy casts give the amyloid reaction—*i. e.*, they assume a mahogany color when treated with a dilute solution of iodopotassic iodide, which changes to a dirty violet upon the addition of dilute sulphuric acid. It should be remembered, however, that this reaction in casts does not necessarily indicate the existence of amyloid disease of the kidneys, as the reaction may be absent in this condition, and present where amyloid degeneration does not exist. This curious phenomenon is usually explained by assuming that such casts have remained in the uriniferous tubules for a long

time, and have there undergone certain chemical changes analogous to the so-called "amyloid metamorphosis" of old precipitates of fibrin. Frerichs has pointed out that fibrin which has remained in the uriniferous tubules for a long time becomes denser and yellowish in appearance, which would explain the fact that these casts are only with difficulty attacked by acetic acid.

The waxy casts, like the brown granular casts, are ultimately supposedly of epithelial origin.

Before leaving this subject it should be stated that "cast-like" formations consisting entirely of amorphous urates are not infrequently encountered in urines, and according to Leube they may be obtained from any urine if it is concentrated in a vacuum at a temperature of 37° to 39° C. Students frequently regard such formations as coarsely granular casts, an error which may be guarded against if the characteristics of hyaline casts set forth above are borne in mind. Such structures are not colored by eosin.

Bacteria (in cases of infectious pyelonephritis), hematoidin, and granular detritus frequently occur grouped in a cast-like manner; their nature is readily ascertained, as in the case of the so-called urate casts just described.

Pseudocasts.—Pseudocasts, consisting of epithelial cells or blood corpuscles and fibrin, are not often found in urinary sediments. The epithelial pseudocasts are probably seen only in cases of desquamative nephritis, and, unlike true casts, are hollow, the epithelium of the uriniferous tubules being thrown off *en masse*. Blood casts consist of fibrin, within the meshes of which red corpuscles are found; these either present a normal appearance or occur as shadows, owing to the fact that their hemoglobin has been dissolved. They are seen whenever extensive hemorrhage has taken place in the renal parenchyma, and are more common than the epithelial pseudocasts.

Cylindroids.—Cylindroids (Fig. 183) resemble hyaline tube casts somewhat in general appearance, but differ from them in being much larger and band-like. Like true casts, they have a uniform breadth, and are often beset with crystals and cellular elements, such as leukocytes, red corpuscles, and epithelial cells. They are readily dissolved by acetic acid, thus differing from the *mucous cylinders* or pseudocylinders (Fig. 184), which may be observed in any urine containing mucus; the latter probably never contain morphological or mineral constituents, and are never of uniform breadth throughout their length. The cylindroids proper are undoubtedly of renal origin and closely related to true casts; formations are indeed not infrequently seen in which a tube cast terminates in a cylindroid at one or both ends.

Clinical Significance of Tube Casts.—Formerly the occurrence of tube casts in urine was held to indicate the existence of nephritis. This view has been abandoned, however, for the same reason which

led to the rejection of the idea that albuminuria invariably indicates Bright's disease (see above).

The statement is frequently made in text-books that tube casts may occur in the urine of perfectly healthy individuals, following severe muscular exercise, cold baths, etc.—in short, stimuli which

FIG. 183.—*a* and *b*, cylindroids from the urine in congested kidney. (v. Jaksch.)

FIG. 184.—Mucous cylinders

may cause the appearance of albumin in apparently normal individuals. It has been indicated elsewhere (see Functional Albuminuria), however, that such stimuli cannot be regarded as "physiological" in every instance, and *the presence of tube casts in the urine similarly should be regarded as a pathological event.* This, however, does not invalidate the now generally recognized fact that a small number of

hyaline and granular casts can be demonstrated in the *centrifugated* urine of many people who are to all intents and purposes in good health.

It is not necessary in this connection to enumerate the various diseases in which cylindruria is observed, as they are the same as those which give rise to albuminuria; and just as a *nephrangiogenic albuminuria* is more frequently observed than a *nephritidogenic albuminuria,* so also is the presence of tube casts in the urine more frequently due to circulatory disturbances than to nephritis. In every case in which tube casts occur in the urine it may be assumed that the accompanying albuminuria is, to a certain extent at least, of renal origin.

Formerly it was though possible to diagnosticate the character of the underlying renal disturbance from the type of casts found in the urine. This, however, is not the case. While, generally speaking, blood and epithelial cells are found in acute and granular casts in chronic processes, there are exceptions so numerous that it would not be safe to follow such a rule. It is remarkable to see the large number and the many varieties of casts which may be found in the urine during the first twenty-four to forty-eight hours after anesthesia, and to observe how rapidly they may disappear, no evidence remaining whatsoever that the renal parenchyma had shortly before been seriously taxed.

Cabot has pointed out the lack of correspondence between the clinical diagnosis of renal disease, as based upon urinary examination and the pathological findings, and has given expression to what many clinicians have previously realized, viz., that neither the diagnosis nephritis nor the type of the renal disturbance can usually be made with certainty in the laboratory. My own experience has led me to the conclusion that so far as cylindruria is concerned the continued presence of hyaline and granular casts, especially of the dark-brown variety, is a symptom of greater gravity than the temporary occurrence of the other types. Hyaline casts *per se* are found under the most diverse conditions. Almost any renal disturbance, whether temporary or permanent, leads to their appearance. Their number is sometimes most remarkable, notwithstanding the fact that no permanent renal damage has been done. Finely dotted and finely granular casts are generally present at the same time.

As the granular cast is generally viewed as a hyaline cast which has been retained in the tubules for a longer time, and, as a result, has undergone changes leading to its granular appearance, it might be inferred that in many temporary disturbances this type is not found. In a general way this is true, but the *occasional* finding of granular casts only should not lead to the diagnosis of a chronic disturbance. They also can appear quite suddenly and disappear almost as rapidly.

Epithelial casts and blood casts are met with in acute processes or in acute exacerbations of chronic processes.

Waxy casts always indicate a chronic or, at least, a subacute process. The fatty casts described by Knoll and v. Jaksch "are most commonly associated with subacute or chronic inflammations of the kidney of protracted course, with a tendency to fatty degeneration of the renal tissue. Postmortem examination has shown that they form most frequently in cases of large white kidney. In some cases in which they were present, however, the organ was found to be more or less contracted; but when this was so, it was invariably far advanced in fatty degeneration." (v. Jaksch.)

It has been stated that from an examination of the renal epithelial cells it is often possible to determine whether an inflammatory process affecting the kidneys is at the same time complicated with degenerative changes. As a matter of fact, the cells found on the tube casts under such conditions no longer present a normal appearance, but are shrunken and atrophied, and in cases of fatty degeneration studded with fatty granules.

The occurrence of *pus casts* presupposes the existence of suppurative inflammation in the kidneys, while the presence of only a small number of leukocytes on hyaline casts may be observed in the ordinary forms of nephritis, and particularly in the acute form.

Cylindroids are present whenever hyaline casts are seen, and have essentially the same import. They are said to occur most frequently in the urine of children.

So far as the constancy is concerned with which tube casts occur in the urine in nephritis, it is well known that in the chronic interstitial form of the disease they, as well as albumin, are frequently absent for a long time, so that it may only be possible to make the diagnosis from the clinical history and the physical signs. It is a well-known fact, moreover, that pathological alterations of the kidneys, particularly in men past middle age, are observed again and again in the postmortem room, where a previous examination of the urine showed no evidence of the existence of renal disease. In the acute and subacute forms of nephritis, as well as in the ordinary parenchymatous form, tube casts are probably always found, and it would further appear that acute circulatory disturbances affecting the renal parenchyma quite constantly lead to both albuminuria and cylindruria.

Within recent years attention has been repeatedly called to the occasional occurrence of cylindruria without albuminuria. Nothnagel first noticed this in a case of icterus. Lüthje observed the same after administering salicylic acid, and Stewart has drawn attention to its occurrence in the early stages of chronic nephritis. I have observed the same after the administration of ether.

SPERMATOZOA

Spermatozoa are frequently observed in the urine of healthy adults, and are quite constantly met with in the first urine passed after coitus or nocturnal emissions, when their presence is, of course, of no significance (Fig. 185).

In females semen may be found in the urine when the external genitals have been polluted during, as well as in the exceptional cases in which connection has been effected by the urethra. From a medicolegal standpoint the discovery of spermatozoa in the urine of women may be of great importance, but otherwise it is, of course, without significance.

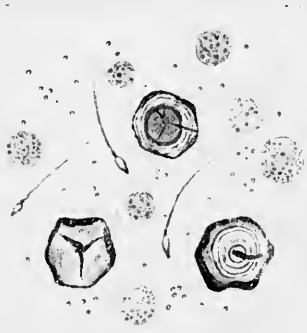


Fig. 185.—Spermatic fluid, showing spermatozoa, corpora amylacea, and lecithin corpuscles.

In pathological conditions spermatozoa are not infrequently found in the urine. In cases of obstinate constipation, owing to pressure of hard, scybalous masses upon the seminal vesicles, a partial evacuation of semen may occur. Horowitz has pointed out that a discharge of semen may be noted in cases of peri-urethral abscess with perforation into the ejaculatory ducts, giving rise to *spermatocystitis*, the condition being due to a tight stricture of the urethra with dilatation beyond the constricted portion. I have observed a case of cystitis in which spermatozoa could almost always be detected in the urine. An operation revealed a tight stricture of the urethra and a sacculated bladder; the constant passage of semen was apparently owing to the irritating action of the ammoniacal urine. In the urine voided during and after epileptic and, more rarely, hystero-epileptic seizures spermatozoa may be found. Such an event is undoubtedly due to muscular spasm, and is identical in origin with the emission of semen observed so frequently in the death agony and during strangulation.

In certain spinal diseases semen may be found in the urine, and Fürbringer relates a case in which, following fracture and dislocation of the vertebral column, with partial destruction of the middle dorsal cord, spermatorrhea associated with partial erection occurred thirty hours later, and continued until death, which took place after three days.

More important is the loss of semen noted in cases of true *spermatorrhea* due to venereal excesses or masturbation, when spermatozoa

may be found almost constantly, and the diagnosis, indeed, will often be dependent upon such an observation.

So far as the question of *sterility* in the male is concerned, reliance should not be placed upon an examination of the urine, but the semen should be obtained as soon as possible after ejaculation, and examined as indicated elsewhere.

PARASITES

Vegetable Parasites.—It has been shown by numerous investigations that bacteria are always present both in the male and female urethra, and that they may *at times* gain entrance to the bladder. The weight of evidence, however, is in favor of the view that the urine *intra vesicam* is under normal conditions free from microörganisms, and that bacteria which may have found their way into the bladder are rapidly killed in healthy individuals. In every urine, on the other hand, that has been exposed to the air, bacteria are always present. Whenever, then, it is desired to determine whether or not the urine of the bladder contains microörganisms, every precaution should be taken to guard against accidental contamination. To this end the following method should be employed. If the patient is a male, he is instructed to hold his urine until a fairly large amount has accumulated. The glans is then thoroughly washed with soap and water, and further cleansed with cotton soaked in mercuric chloride solution (1 to 1000). The fossa navicularis is also thoroughly cleansed with the same solution. The urine is then voided under as great pressure as possible. The first portion (about 100 c.c.) is thrown away, and the second received in a sterilized vessel, when cultures should be made at once, agar or gelatin plates being inoculated with 1 or 2 c.c. of the urine. In the female the vulva is cleansed with soap and water, and the urethral aperture disinfected with bichloride solution. After then washing with sterilized water and drying with sterilized cotton the urine is evacuated through a sterilized metallic or glass catheter, and received in a sterilized vessel. Brown describes the method which is in use in Dr. Kelly's department at the Johns Hopkins Hospital as follows: The external urethral orifice being carefully cleansed with mercuric chloride solution, followed by sterile water, a sterilized glass catheter, whose external end is covered by a sterile rubber cuff, extending several centimeters beyond the end of the catheter, is introduced, the fingers of the operator being allowed to touch only the distal end of the rubber cuff. The urine is allowed to flow for a short time, when the rubber cuff is pulled off by traction on its distal end. A small amount of urine is then collected in a sterile test-tube, and the cotton plug immediately inserted. Brown states that an extended series of

experiments with normal urines has shown that this method is absolutely reliable.

Of the bacteria which may be found in every urine that has been exposed to the air, the *Micrococcus ureæ* is of special interest, as ammoniacal fermentation is largely due to its presence. When fermentation has commenced, it is readily recognized, occurring in almost pure culture upon the surface of the urine, mostly in the form of characteristic chains. The individual coccus is colorless and quite large, so that it may be mistaken by beginners for a blood shadow.

It is a common error to infer from the occurrence of ammoniacal decomposition very soon after micturition that this has already begun in the bladder. It should be remembered that urine may undergo fermentation, particularly in warm weather, shortly after having been voided, and especially if the vessel employed is not perfectly clean and the urine has been exposed to the air. The diagnosis of ammoniacal fermentation in the bladder should hence only be made when the presence of ammonia can be demonstrated in the urine immediately upon being voided.

Under pathological conditions various pathogenic bacteria may be found in the urine. Pyogenic cocci are especially prone to settle in the kidneys, and there give rise to focal inflammations; but even in the absence of such lesions they are frequently found in the urine. In all forms of infectious nephritis an abundant elimination of bacteria may generally be observed. Von Jaksch states that in erysipelas the bacteriuria and nephritis disappear, together with the cessation of the disease, and in various suppurative processes taking place in the body the specific bacteria disappear from the urine within twenty-four to forty-eight hours after evacuation of the pus.

Most interesting observations on the occurrence of bacteria in the urine of nephritic patients have been reported by Engel: 31 cases were examined. In 16 the Staphylococcus albus and aureus were found, in 8 pyogenic streptococci, in 4 the tubercle bacillus, in 5 the Bacillus coli communis, and in 1 the typhoid bacillus, while negative results were obtained in only 2 instances. In the same series Engel also found a pyogenic coccus in 17 cases. This coccus was larger than the known forms; it could be stained according to Gram's method, and did not liquefy gelatin. Intravenous injections of large numbers of the organism caused nephritis in rabbits.

In pneumonia and pneumococcus infections in general the corresponding diplococcus may be found, and in erysipelas and streptococcus infections streptococci. In scarlatina streptococci have been found in a large percentage of cases; the urine was then usually albuminous.

In cases of pyelitis the colon bacillus is very frequently met with. It is usually present in pure culture, but may be associated with

other organisms, notably the Proteus vulgaris and staphylococci. These latter may, however, also be met with in pure culture.

In *renal tuberculosis* the corresponding bacilli appear very early and are always present in the pus and debris. The search for them is usually very tedious, and small numbers only are found, but at times they are very numerous. To demonstrate their presence the urine is allowed to settle for twelve hours. Slides are prepared, which must be free from fat. To this end they are boiled for thirty minutes in a strong solution of caustic soda and then washed for an equal length of time in running water, after which they are wiped dry. Two drops of the sediment are placed on each one of six slides. They are placed on a frame some ten inches above a Bunsen burner, which is kept low, so as to insure slow evaporation. When thoroughly dry they are fixed by passing through the flame of the burner and placed for five minutes in 5 per cent. acid (HCl) alcohol to dissolve the urinary salts. After washing in water the specimens are then stained as usual. Using Gabbett's method, they are stained for ten minutes with the carbol fuchsin solution and then decolorized with the acid methylene blue. If but little pus is present the urine may be centrifugalized.

Using the above method, Walker states that he could demonstrate tubercle bacilli in each case in which tuberculosis was afterward found.

In doubtful cases animal inoculation should be practised. The urine is received by the catheter into a sterile bottle, the first portion being allowed to escape. After twelve hours the supernatant fluid is poured off and the sediment drawn into a sterile hypodermic syringe. The material is injected into the subcutaneous tissue of the back of a guinea-pig. If tubercle bacilli are present tuberculosis should develop in from three to five weeks, but may occur even after two weeks.

Intraperitoneal injections may also be practised, although one is more apt to lose the animals from incidental infections before tuberculosis may become manifest. It is said that such secondary organisms may be eliminated by heating the material for ten minutes at 60° C. The appearances seen at autopsy are very characteristic. The spleen, lymph glands, and liver show marked lesions. In cases where death occurs rapidly (in two weeks) miliary tubercles will be seen all over the liver and spleen, while the lymph glands are only moderately enlarged. In less active cases the lymphatic picture is most pronounced; axillary, cervical, and peritoneal glands are very much enlarged and the spleen may be transformed into one huge, caseating mass.

On repeated occasions *smegma bacilli* have been mistaken for tubercle bacilli. They are most frequently met with in women; this, however, only in non-catheterized specimens. Greenbaum

PLATE XXVI

Urethral Discharge from a Case of Gonorrhea, showing Gonococci Inclosed in Pus Corpuscles and Lying Free in the Discharge.

Stained with Methylene Blue. (Personal Observation.)

states that after thoroughly wiping the meatus and introducing a sterile catheter he never found them.

In the male, confusion with the smegma bacillus is less likely to occur, and if pains are taken to wash the glans and to irrigate the urethra, as advised by Young and Churchmann, it may be eliminated altogether as a disturbing factor. To this end the following technique is recommended: The foreskin, if present, is rolled back and held back by the patient. The glans is thoroughly scrubbed with soap and water. This must be done with great care, using very large amounts of water for the rinsing. An irrigator is filled with sterile water and the nozzle attached. This is made from a piece of small-caliber glass tubing with a circumference of a 15 F. sound and about seven and one-half inches long. The sharp edges of one end are rounded by fusing in the Bunsen flame. The other end is inserted into a piece of rubber tubing of the proper diameter to make a snug fit. The glass tube is pushed into the rubber tube about one inch, leaving about six and a half inches free. A rubber guard (conveniently made from one-half of a rubber ball) is fitted snugly over the rubber tubing near its end, about six and one-half inches from the fore end. The nozzle is connected with the tube of an ordinary irrigator, hung high enough to give a good pressure, the patient being instructed to keep his sphincter urethræ closed during the procedure. The water is then allowed to flow, the glans and meatus well rinsed with it, and the nozzle gradually inserted and passed back to the triangular ligament (the tube is long enough to reach this), the stream flowing constantly during its insertion and withdrawal. A quart of irrigating fluid is used (Young and Churchman).

Whether any reliable staining method exists whereby the smegma bacillus can be definitely distinguished from the tubercle bacillus seems doubtful. Trudeau suggests staining in the usual way with carbol fuchsin, to decolorize with 25 per cent. nitric acid, then to wash and place the specimens for two minutes in 95 per cent. alcohol, and to counterstain with blue. But he states that he does not find any method reliable in all cases, and in doubtful cases advises inoculation of a guinea-pig.

Of great interest is the frequent occurrence of the *typhoid bacillus* in the urine of typhoid fever patients. (See section on Typhoid Fever.) The bacillus may be isolated and identified according to the usual methods. (See Blood and Feces.)

In cases of *paratyphoid* fever the corresponding bacilli may be found in the urine.

Gonococci may be found in urinary sediments inclosed in pus cells, and can be demonstrated by preparing smears and staining with a basic dye or with the eosinate of methylene-blue solution. In the so-called gonorrheal threads they can often be found years after the infection (Plate XXVI).

In cases of *bubonic plague* Kitasato's coccobacillus may be found in the urine.

In cases of cystitis a great variety of microörganisms has been met with in the urine. Among the more important may be mentioned the Staphylococcus aureus, albus, and citreus, streptococci, the Bacillus coli communis, the Bacillus pyocyaneus, the Bacillus typhosus, the Proteus vulgaris, the gonococcus, etc. In many cases of cystitis organisms are found, moreover, which are apparently non-pathogenic, and are capable of causing the formation of hydrogen sulphide from certain sulphur bodies of the urine. (See Hydrothionuria.)

In conclusion, reference should be made to the occasional occurrence of a form of bacteriuria which is not associated with any pathological process, and has hence been termed *idiopathic bacteriuria.* Of its causation and significance nothing is known, but it is possible that in these cases a few bacteria enter the bladder either through the anterior rectal wall or are eliminated through the kidneys from the blood current. Finding a suitable medium for their growth in the urine they here multiply and may thus be constantly present. The diagnosis "idiopathic bacteriuria" should, of course, only be made if every possible source of contamination of the urine can be definitely excluded.

Urines containing bacteria in large numbers are always cloudy, and usually present an acid reaction when voided unless cystitis exists at the same time. Attention is directed to their presence by the fact that such specimens cannot be cleared by simple filtration.

Actinomyces kernels may be observed in the urine when the disease in question has attacked the genito-urinary tract or when the organism has found its way into the urine from other organs.

Yeast cells in large numbers are usually only seen in urines containing sugar. When a chemical examination has not been made their demonstration will be of importance, as suggesting the possible existence of glucosuria.

Molds are usually seen in old diabetic urines after alcoholic fermentation has taken place, but they may also occur, though far less frequently, upon the surface of putrid urines that have contained no sugar.

The urinary *sarcina* which is at times met with is smaller than the sarcina of the gastric contents, but closely resembles it in appearance. It is of no clinical significance.

Animal Parasites.—The organism which Hassal saw in a urine that had been "freely exposed to the air" and was alkaline, and which he termed Bodo urinarius, was in all probability an infusorial monad. Salisbury was the first to point out that the *Trichomonas vaginalis* of Donné may at times occur in the bladder, but he gave no detailed account of his cases. Künstler, Marchand, Miura, and

Dock subsequently reported cases in which flagellate protozoa were found, and modern research leaves no doubt that the organisms described by these observers are identical with the trichomonas of Donné. In Miura's case the habitat of the parasite was the urethra, and an examination of the patient's wife revealed the presence of similar organisms in the vagina. Künstler's case was one of pyelitis following cystotomy. Marchand's patient had a fistula in the perineum following suppuration in the pelvis, of unknown origin; cystitis did not exist. Dock's case was associated with hematuria. During the past few years I have seen the same organism in several cases. Most of them were women, and I have no doubt that the parasite found its way into the bladder from the vagina, where it could be demonstrated in 2 instances. Curiously enough a history of hematuria was obtained from 4 patients. In 2 cases the urine contained blood at the time of the examination. In 1 case there was evidence of nephritis; cystitis did not exist. The number of the parasites was variable, and sometimes quite large.

Bälz observed innumerable amebæ in the turbid urine of a girl the subject of phthisis, which he described as being of larger size than the Amœba coli. Jürgens found amebæ in a patient suffering with a tumor of the bladder. Wijuhoff reports their presence in the urine in 4 cases. Posner cites 1 instance and Musgrave and Clegg another, the latter a case of hemorrhagic cystitis.

In cases of bilharziasis the ova of the parasite (see Blood) are encountered in the urine together with blood. Sometimes the entire bulk of the urine is blood-tinged, but more often only the last few drops contain blood, and in these last drops the eggs of the parasite will also be found. In doubtful cases it is always best to examine this portion. The eggs are readily seen with a low power. (See Fig. 54.)

Filaria embryos may be found in the urine in cases of filarial chyluria. They should be looked for in the coagulum, a bit of which is teased out and pressed between two slides.

Billings and Miller have reported the possible occurrence of the Anguillula aceti in the urine, in cases in which the urine is collected in bottles that had contained old vinegar. The worm very closely resembles the Anguillula stercoralis. Stiles has made a similar observation.

Echinococcus hooklets and fragments of cysts may also be found, and in rare instances ascarides find their way into the urinary passages. Bothriocephalus linguloides (Leuckart) was found in the urine in a case occurring in Eastern Asia. Eustrongylus gigas is likewise found very rarely. Moscato records one case in which chyluria existed at the same time. In Clark's case, which was reported in the United States, the passage of the worm was accompanied by hematuria.

TUMOR PARTICLES

Tumor particles are so rarely seen in the urine that a detailed account of their occurrence may be omitted, particularly as it is seldom possible to base the diagnosis of tumor upon the presence of fragments in the urine, the clinical history and the physical signs being usually sufficient to reach a satisfactory diagnosis.

FOREIGN BODIES

Of foreign bodies which may be found in the urine may be mentioned particles of fat, fibers of silk, linen, and wool, etc.; in short, material the presence of which is owing to the use of unclean vessels for the reception of the urine. Fecal matter may be passed by the urethra; such an occurrence, of course, indicates the existence of an abnormal communication between the bowel and the urinary passages. Hair derived from a dermoid cyst may similarly be found. In hysteria foreign bodies of almost any kind, such as hair, teeth, fish-bones, wood, etc., and even snakes and frogs, may be shown the physician as having been passed in the urine. I had occasion to examine "gravel" "passed" from time to time by an hysterical patient in large amounts, "every attack being accompanied by agonizing pains shooting down into the lower abdomen;" the gravel upon examination proved to be mortar obtained from the cellar of the patient's house. Still more recently I was shown a urine of a bright eosin color, and was told that it had been voided by a girl of twelve. The color was manifestly "unnatural," and proved to be referable to the addition of paint from the child's paint box, her object being to attract attention and to be excused from going to school.

CHAPTER VIII

TRANSUDATES AND EXUDATES

In health the so-called serous cavities of the body contain very little fluid, and quantities sufficient for analytical purposes can normally only be obtained from the pericardial sac. In pathological conditions, on the other hand, large accumulations of fluid may be observed, not only in the serous cavities, but also in the areolar connective tissue, beneath the skin, and beneath the muscles. When due to circulatory disturbances, or a hydremic condition of the blood, such accumulations of fluid are spoken of as *transudates*, while the term *exudates* is applied to similar accumulations of inflammatory origin.

Clinically, it is frequently difficult to distinguish between transudates and exudates, and large ovarian, pancreatic, and hydatid cysts, as well as cystic kidneys, may at times be mistaken for ascites. In such cases a careful chemical and microscopic examination of the fluid in question may be of value. Very frequently, moreover, it is possible *only* in this manner to determine the nature of the disease, and *the free use of the trocar and the aspirating needle in diagnosis cannot be too strongly advocated.*

TRANSUDATES

General Characteristics.—Transudates are usually serous in character, when they present a light straw color; at times, however, owing to admixture of blood, they have a reddish tinge, and are then said to be hemorrhagic; in rare instances they are chylous.

Specific Gravity.—The specific gravity varies somewhat according to the origin of the fluid, but is usually lower than that of serous exudates occurring in the same cavities—one of the most important points of difference between the two kinds of fluid. Thus in acute pleurisy the specific gravity of the exudate is usually higher than 1.020; and in chronic pleurisy, if an accumulation of pus exists at the same time, higher than 1.018, reaching even 1.030. In transudates into the pleural cavity, on the other hand, referable to circulatory disturbances, for example, as in cases of hepatic cirrhosis or cardiac insufficiency, the figures obtained are usually lower than 1.015. Transudates of peritoneal origin similarly present a specific gravity

varying between 1.005 and 1.015, while that of exudates frequently reaches 1.030.

As the chemical composition, in so far as the mineral constituents and extractives are concerned, is practically the same in both classes of fluid, the difference in the specific gravity appears to be essentially due to the amount of albumin present, viz., serum albumin and serum globulin. It may be demonstrated, as a matter of fact, that exudates contain far more albumin than transudates, the amount varying between 4 and 6 per cent. in the former, as compared with 1 and 2.5 per cent. in the latter. The largest amounts of albumin in transudates are found in those of pleural origin, while in edema not more than 1 per cent. is usually present.

Reuss suggests the following formula for the purpose of determining from the specific gravity the amount of albumin in transudates and exudates:

$$E = \tfrac{3}{4}\,(S - 1000) - 2.8$$

in which E indicates the percentage amount of albumin and S the specific gravity taken by means of an accurate urinometer.

Subsequent examinations have shown, however, that this formula is not applicable, since the amount of albumin is not strictly proportionate to the specific gravity.

Since the use of Esbach's albuminimeter is totally insufficient for this purpose, Strubell, Reiss, Strauss and Chajes, and Engel suggest a refractometric examination, which depends essentially upon the amount of albumin present, but even with this method the results are not always satisfactory. Engel lauds it, however, nevertheless. An analysis of his data follows:

	Pleura.	Abdomen.	Pericardium.
Nephritic transudates	1.3375	1.3374	1.3398
	1.04 per cent.	0.98 per cent.	2.29 per cent.
Cachectic transudates	1.3385	1.3382	1.3398
	1.59 per cent.	1.42 per cent.	2.29 per cent.
Static transudates	1.3392	1.3398	1.3405
	1.97 per cent.	2.29 per cent.	2.66 per cent.
Pleuritic exudates	1.3446		
	4.89 per cent.		
Peritoneal exudates		1.3445	
		4.84 per cent.	
Pericardial exudates			1.3460
			5.64 per cent.

The upper average figures indicate the refractometric co-efficient, and the figures below the corresponding amount of albumin, as calculated from Reiss' tables. For a detailed description of the method the reader is referred to Reiss' paper.[1]

[1] Arch. f. exper. Pathol. und Pharmak., vol. li.

The fact that transudates do not coagulate spontaneously in the absence of blood may further serve to distinguish them from exudates, in which a coagulum is frequently observed after standing for twenty-four hours. Not much reliance should be placed upon this point of difference, however, as exudates likewise do not always coagulate, and clotting of transudates in the presence of blood may take place within the body.

Chemistry of Transudates.—An idea of the chemical composition of the various forms of transudates may be formed from the following tables, taken from Hoppe-Seyler and Hammarsten, the figures corresponding to 1000 parts by weight of fluid; the specimens were taken from one individual:

	Pleura.	Peritoneum.	Edema of the feet
Water	957.59	967.68	982.17
Solids	42.41	32.32	17.83
Albumin	27.82	16.11	3.64
Ethereal extract	14.59	5.27	0.50
Alcoholic extract			3.71
Aqueous extract		10.94	1.10
Inorganic salts			9.00
Errors of analysis			0.12

Analysis of Hydrocele Fluid

Water	938.85
Solids	61.15
Fibrin (formed)	0.59
Globulins	13.52
Serum albumin	35.94
Ethereal extract	4.02
Soluble salts	8.60
Insoluble salts	0.66
Sodium chloride	6.19
Sodium oxide	1.09

Sugar and uric acid in small amounts are also, as a rule, found in transudates, and in one case of hepatic cirrhosis Moscatelli succeeded in demonstrating the presence of allantoin. Von Jaksch states that he has frequently been able to demonstrate the presence of urobilin in both transudates and serous exudates, even though red blood corpuscles and blood-coloring matter in solution were absent. Stich also reports that in the ascitic fluid removed during life from a patient with hemorrhagic nephritis, urobilin was present. Peptone is never found; and Pajikull states that nucleo-albumin is not present in transudates of non-inflammatory origin. Hammarsten, together with Pajikull, could, however, demonstrate an albuminous substance in transudates which was regarded as a mucoid and which is present in exudates in small amounts only. It is rich in reducing substance and contains more nitrogen than the true mucins.

Microscopic Examination.—Upon microscopic examination only a few isolated leukocytes and endothelial cells from the serous surfaces

and undergoing fatty degeneration are usually seen. Mast cells and eosinophilic leukocytes have been observed in the ascitic fluid in cases of myelogenous leukemia. Charcot-Leyden crystals were present at the same time. In cases in which the transudates have been confined for a long time, plates of cholesterin are frequently found. They are especially abundant in hydrocele fluid. Amebæ have been found by Miura in the ascitic fluid of a woman afflicted with an abdominal tumor; at the same time they were present in the stools. Leyden and Schaudinn likewise met with ameboid bodies in the ascitic fluid obtained from two cases of abdominal tumor. The technique which should be employed in the microscopic examination of transudates is described below.

EXUDATES

Exudates may be serous, serofibrinous, hemorrhagic, seropurulent, purulent, putrid, chylous, or chyloid. Of these, the seropurulent, purulent, and putrid types are manifestly of inflammatory origin, while in the case of the serous, serofibrinous, and hemorrhagic forms it may at times be difficult to determine whether the fluid represents a transudate or whether it is an exudate. A detailed chemical and microscopic examination may then be necessary.

Serous Exudates.—Serous exudates are clear, of a light straw color, and present a specific gravity which usually exceeds 1.018 (1.012 to 1.024). There is a large amount of fibrin and of albumin. If blood corpuscles are present in sufficient numbers to impart a distinct red color to the fluid it is termed *hemorrhagic;* the color may then vary from a light pink to a dark red. On standing, even the purely serous exudates generally undergo a certain degree of coagulation, which becomes more marked in the presence of blood; exceptions, however, do occur. Most important is the microscopic examination of the exudates. Generally speaking, the same methods are here employed as in the case of the blood, but the interpretation of the findings is not always easy. This is largely owing to the fact that the leukocytes often show evidence of degeneration, and that the fluid may contain endothelial cells in addition to the morphological elements of the blood, which further increases the difficulties attending a proper classification. (See Pus.) The principal point at issue in the study of the cellular elements of exudates is the question as to the predominance of either lymphocytes or of polynuclear neutrophilic leukocytes. Widal and his collaborators, more especially, have pointed out that whereas in exudates of non-tuberculous, acute inflammatory origin the polynuclear neutrophilic leukocytes predominate, the lymphocytes prevail in the chronic tuberculous forms. His observations have, on the whole, been confirmed by numerous investigators, and the importance of *cytodiagnosis* in pleuritic effusions

more especially is now well established. From the available data we may formulate the following conclusion: In the very earliest stages of tuberculosis involving the serous membranes there is found a variable number of neutrophilic leukocytes in addition to lymphocytes and endothelial cells. Very soon, however, the neutrophiles diminish, and in the later stages the lymphocyte is the predominating cell. Generally speaking, the percentage of lymphocytes in tubercular pleurisies ranges from 50 to 98, increasing as the disease continues.

In pleuritic effusions due to the pneumococcus and to streptococci during the serous stages, the neutrophilic leukocytes outnumber the lymphocytes. (Average in postpneumonic cases, 71.7: variations from 58 to 92.5 per cent.). In the pneumococcic cases, moreover, it is common to meet with large numbers of endothelial cells, sometimes containing polynuclear leukocytes and red cells in their interior.

In cases of traumatic and aseptic pleurisy, in association with diseases of the heart and kidneys, large endothelial cells are seen which often present most grotesque appearances, occurring either singly or in groups of two, three, four, or more; while the occurrence of large numbers of such cells has been regarded as characteristic of transudates, Carter has shown that in these cases also there may be a lymphocytosis of from 86 to 100 per cent.; so that confusion may arise in differentiating these cases from tubercular pleurisy. The low specific gravity—average about 1.008—and the small amount of fibrin and albumin in the transudates will, however, aid in arriving at a conclusion.

French writers also describe a pleural eosinophilia in which large numbers of eosinophilic cells—6 to 54 per cent.—are found in the effusion, while in the circulating blood their number is not increased. Ravaut reports 4 cases of this kind. In 1 the effusion occurred secondarily in the course of syphilis; in the second in a case of typhoid fever; the third was a case of phthisis, while in the fourth no diagnosis was made. I have recently seen a case of this kind (probably tubercular) with 10 per cent. of eosinophiles, 4 per cent. neutrophiles, 83 per cent. of small mononuclears, and 2.4 per cent. of large mononuclears in the exudate, and 3.5 per cent. of eosinophiles, 42 per cent. of neutrophiles, 36 per cent. of small mononuclears, and 18 per cent. of large mononuclears in the blood.

Carter reports 2 cases of pleural effusion, referable to pistol-shot wounds of the chest walls, in which the eosinophiles numbered 70.2 and 87.8 per cent. respectively.

Mast cells are rarely seen in pleuritic effusions, and it has been observed that their granules are then quite readily soluble in water, so that they cannot be demonstrated with aqueous solutions of the usual dyes. Wolf notes a case in which the mast cells constituted about 10 per cent. of the total number of leukocytes.

Whether or not the conclusions which have been reached regarding the meaning of the prevalence of certain cell forms in pleural effusions can be directly applied in the case of peritoneal effusion remains to be seen. From the available data it appears that the indications are not so direct. But, generally speaking, endothelial plaques control the picture in ascites of mechanical origin, while lymphocytes predominate in tubercular peritonitis and in peritoneal carcinoma. The occurrence of large vacuolated cells is suggestive of a cyst accompanied by ascites (ovarian cyst).

The same considerations apply to the cytological study of joint effusions. Widal reports that in 3 cases of acute rheumatism he found polynuclear leukocytes in the serous exudate, while they were absent in traumatic cases of arthritis. As the result of an examination of 30 hydroceles, Marchetti concludes that lymphocytes and epithelial cells predominate without exception.

Of the cytological findings in the cerebrospinal fluid a detailed account will be given later.

Generally speaking the cytological factor does not seem to depend so much upon the anatomical localization of the morbid process as upon its duration and the character of the pathogenic agent. An acute process (pneumococci, streptococci) call forth a lymphocytosis of brief duration, which is followed sooner or later by a granulocytosis, while a less intense stimulus, and one acting more slowly (tubercle bacillus) leads to a persistent lymphocytosis. The possibility that a stimulus of the latter order may act with undue virulence and intensity, and that one of the first type may be exceptionally mild and delay the occurrence of granulocytosis, should, however, be borne in mind.

Very important also is the study of the cellular elements which are found in serous exudates in cases of malignant disease of the serous membranes. Difficulty may here be encountered in the interpretation of the cellular findings, for on the one hand it is often difficult to distinguish the endothelial cells from leukocytes, as they take on phagocytic activity and often present the most bizarre forms. The nucleus, which is normally centrally located, takes up an excentric position, and inclosed within the cell we may find leukocytes and red cells. On the other hand, it is impossible by simple inspection to distinguish normal endothelial cells from *cancer cells*. In cases of doubt it is well to ascertain whether the epithelial elements give the glycogen reaction. Quincke has pointed out that normal endothelial cells do not contain glycogen, and that a marked iodine reaction is very suggestive of carcinoma. Wolff, however, suggests that this test is probably not specific, and cites two instances in which he obtained a positive glycogen reaction, although a tumor did not exist. More important is the presence of mitoses. In nonmalignant exudates epithelial cells never present evidence of mitosis,

while in cases of tumor this may be found. Rieder regards their occurrence as pathognomonic of malignant disease. Commonly the mitosis is atypical; the division of the nucleus is not followed by a division of the cell; the chromosomes are short and show no polar or equatorial arrangement.

In cases of neoplasm Quincke has also drawn attention to the occurrence of large numbers of fat droplets in the fluid, which may attain a diameter of from 40 to 50μ. At times, however, the fat droplets are so small and so numerous as to give a chylous appearance to the exudate. At other times a similar appearance is due to the presence of minute albuminous granules, which may be distinguished from fat by their insolubility in ether and the fact that they are not stained with the common fat dyes, such as Sudan, scarlet-R, and alkanin. The occurrence of numerous fatty acid crystals, arranged in groups, should also excite suspicion of a neoplasm.

Should bits of tissue be obtained, a positive diagnosis of malignant disease may, of course, be made by the usual methods. Such particles should be placed at once in absolute alcohol or formalin.

Crystalline elements are not usually seen in serous or hemorrhagic exudates; at times we meet with platelets of cholesterin.

Technique.—In every case the fluid should be examined as soon after puncture as possible; if this cannot be done at once, coagulation may be prevented by the addition of sodium citrate. The material is then placed in the ice-box until a sediment has collected, or this may be obtained at once by centrifugation, new portions of fluid being repeatedly used and the sediments combined. Cover-glass preparations may then be conveniently made, or smears on slides exactly as in the case of blood, care being taken to do as little injury to the cellular elements as possible. The smears should be very thin, so that the specimens will dry rapidly and but little chance given for the cells to contract beyond their usual size. Subsequent treatment will depend upon the special points which are to be elicited. Unfortunately the leukocytes are often much changed so that their classification may be attended by considerable difficulties. The polynuclear elements may appear mononuclear, and not infrequently the neutrophilic granules can no longer be demonstrated. (See Pus.) For this reason the triacid stain is not to be recommended for routine work; the eosinate is much better and will furnish as satisfactory results as can be obtained with a panoptic dye. Successive staining with eosin and methylene blue sometimes gives better results than a polychrome dye. Care should be had not to diagnosticate eosinophilia from the fact that cell granules are stained red, as the neutrophilic granules of degenerating cells are commonly amphophilic, viz., they stain both with acid and neutral dyes; account must be taken of the size of the granules and the general structure of the cell. To differentiate pseudolymphocytes from true

lymphocytes, Pappenheim's methyl-green pyronin may be employed, though it is not absolutely specific; still it will be found that even though the protoplasm of other cellular elements may take the red color of the pyronin, the intensity is distinctly less than in the case of the lymphocytes proper.

Pappenheim's Method.—The stain is composed of a concentrated aqueous solution of methyl green to which pyronin is added until the solution just begins to turn blue, viz., about 1 part of pyronin for 3 to 4 parts of methyl green. Stained in this manner, the basophilic protoplasm of the lymphocytes is colored a fine dark carmine red, while the protoplasm of all other cells is stained a more or less pale brownish or reddish yellow, or remains colorless. Pappenheim regards this stain as essentially specific for the lymphocytes, but admits that it also stains in a similar manner the young erythroblasts that are poor in hemoglobin. The difference can be recognized from the character of the nuclei and the fact that the margin of the lymphocytes very commonly appears shaggy, while that of the erythroblasts is smooth and homogeneous.

To study *mitoses*, hematoxylin and eosin may be employed, or the Romanowsky method in one of its various modifications.

The glycogen reaction is demonstrated as in the case of the blood.

Bacteriological Examination of Exudates.—In a measure the bacteriological examination of exudates has been supplanted by the cytological study, as outlined above; especially as the bacteriological examination has been notoriously unsatisfactory in the most important group of effusions, viz., in those of tubercular origin. It is now known that *all* exudates gradually become free from bacteria, even though at first they may have been caused by bacterial activity. As a result it is no longer justifiable to conclude that a process is tuberculous because bacteriological examination of the exudate has given no positive result. If it is desired to cultivate organisms that may be present, it is well to make a bouillon culture in every case so as to eliminate the bactericidal properties of the exudate as much as possible. In any event it is well to centrifugate the fluid in a sterile tube and to use the sediment for inoculations. The organisms which are most likely to be encountered are the pneumococcus, the various staphylococci, streptococci, and more rarely the colon bacillus and the typhoid bacillus.

Inoscopy.—Jousset recommends the following procedure for the purpose of demonstrating tubercle bacilli in exudates: The fluid is allowed to clot spontaneously or by adding a little horse serum. The clot, which is supposed to contain most of the organisms, is pressed out, torn into fragments, and placed in about 10 c.c. of a digestive mixture of the following composition: pepsin, 1 to 2 grams; glycerin, 10 c.c.; 40 per cent. solution of hydrochloric acid, 15 c.c.; sodium fluoride, 3 grams; water, 1000 c.c. The material is left in the incu-

bator for three to four hours, then centrifugalized and smears prepared from the sediment and stained as usual. Jousset claims to have obtained very good results in this manner, while others are less enthusiastic.

More recently Zebrowski has suggested the following method as more likely to lead to satisfactory results: Coagulation of the fluid is prevented by the addition of an equal volume of a 0.5 per cent. solution of sodium fluoride. The mixture is set aside in a cool place until the following day, when it is thoroughly centrifugated and smears made from the sediment and stained as usual.

With this method Zebrowski claims to have found tubercle bacilli in 83 per cent. of secondary and 55 per cent. of primary pleurisies.

More satisfactory than either method possibly is the animal experiment, to which end a large quantity of the fluid is centrifugalized and the sediment injected into the peritoneal cavity of a guinea-pig, as in the case of the urine (which see).

Chemistry of Exudates.—According to Moritz, an albumin is found in exudates that can be precipitated with acetic acid and which is absent in transudates. He regards this as serum globulin which has undergone a change as a result of the inflammatory process. According to Matsumoto, on the other hand, the substance in question represents a mixture of fibrinoglobulin, euglobulin, and a small amount of pseudoglobulin; in the filtrate, however, there is also some fibrinoglobulin (fibrinogen) and euglobulin. He suggests that this last circumstance is probably referable to the small amount of salt in exudates and that in the first instance the pseudoglobulin is probably carried down mechanically.

More recently Umber has studied the body in question and arrived at the conclusion that it belongs to the mucins. To its presence the mucinous character of such fluids is due. It is precipitated by the addition of acetic acid and is insoluble in an excess of the reagent unless the acid is present in great concentration. The body has markedly acid properties and is not coagulated by heat. It differs from the known mucins in the presence of a very small amount of reducing substance, which can only be demonstrated by special methods. It contains about 14 per cent. of nitrogen and no phosphorus. In neutral and feebly acid solution the substance does not coagulate (thus differing from the globulins). The same body apparently was found by Salkowski in an exudate into the hip-joint. Umber calls this substance *serosamucin*. Its amount is less than 0.5 per cent.

According to Umber and Stähelin the serosamucin is essentially found in exudates referable to inflammatory processes or associated with newgrowths. In transudates, as Runeberg already pointed out, only a very slight turbidity results upon the addition of acetic acid, and not in all cases, moreover; so that a well-marked reaction, viz., a marked precipitation upon the addition of acetic acid to the

point of a distinctly acid reaction may be regarded as a valuable sign in the diagnosis between transudates and exudates. I append some of the results obtained by Umber:

Ascites	No. of cases.	Serosamucin.
Hepatic cirrhosis	6	0
Hepatic cirrhosis with chronic nephritis and phthisis	1	0
Nephritis	1	0
Mitral disease	3	0
Pleural Exudates		
Degeneratio cordis and nephritis	2	0
Myocarditis	1	0
Hepatic cirrhosis	1	0
Lymphosarcoma (pleura intact post mortem)	1	0
Carcinoma mammæ with pleural metastases	1	+
Tuberculosis of pleura	1	+
Pleuritis exsudativa acuta	1	+
Pleuritis and pericarditis	1	+

Of the common albumins we meet with traces of fibrinogen and with fairly large amounts of globulin and serum albumin. Their percentage may at times not appear so very large, but considering the large amount of fluid and the rapidity with which it may accumulate, it is clear that the loss of nitrogen to the body in this form may be very considerable. Umber showed that in one of his cases 5000 grams of albumin, representing about 15,000 grams of muscle tissue, were lost within a year.

In addition to the serosamucin and the common albumins mentioned, some exudates may possibly also contain small amounts of a nucleo-albumin, as is suggested by the findings of Pajikull. Should ovarian cysts have ruptured into the peritoneal cavity, we may further find both pseudomucin and paramucin (which see).

Of interest further is the fact that Umber succeeded in demonstrating the existence of autolytic processes in exudates. He found both albumoses and mono-amino acids, viz., leucin and tyrosin.

Coriat has reported a case of polyneuritic delirium, in which pleurisy with effusion developed. In the effusion he could demonstrate a peculiar albuminous substance, which he regards as identical with Bence-Jones' albumin; in the urine this substance could not be found.

PUS

General Characteristics of Pus.—If pus, which usually presents a color varying from yellowish gray to greenish yellow, is allowed to stand for a time a liquid gradually appears at the top, and increases in amount until it is finally possible to distinguish two

distinct layers: the one above, the pus serum; the other at the bottom, the pus corpuscles. Upon the number of the latter the consistence as well as the specific gravity of the pus is dependent. This may vary between 1.020 and 1.040, with an average of 1.031 to 1.033. Fresh pus has always an alkaline reaction, which may become neutral or slightly acid upon standing, owing to the development of free fatty acids, glycerin-phosphoric acid, and lactic acid. The color of pus serum may be a light straw, a greenish or a brownish yellow.

Chemistry of Pus.—The chemical composition of pus serum and pus corpuscles may be seen from the following tables:

Analysis of Pus Serum

	I	II
Water	913.70	905.65
Solids	86.30	94.35
Albumins	63.23	77.21
Lecithin	1.50	0.56
Fat	0.26	0.29
Cholesterin	0.53	0.87
Alcoholic extract	1.52	0.73
Aqueous extract	11.53	6.92
Inorganic salts	7.73	7.77

Analysis of Pus Corpuscles

	I	II
Nuclein	342.37	
Insoluble matter	205.66	673.69
Albumins	137.62	
Lecithin	143.83	75.64
Fat		75.00
Cholesterin	74.00	72.83
Cerebrin	51.99	102.84
Extractives	44.33	

Albumoses are usually present, and are derived from the pus corpuscles. Leucin and tyrosin are likewise frequently met with in the pus of old abscesses; and fatty acids, urea, sugar, glycogen, biliary pigments and acids (in catarrhal jaundice), acetone, uric acid, xanthin bases, cholesterin, etc., have occasionally been observed.

Microscopic Examination of Pus.—Leukocytes.—If a drop of pus is examined with the microscope it will be seen to contain innumerable leukocytes, many of which in perfectly fresh pus exhibit ameboid movements. The cells in question are usually almost altogether of the neutrophilic variety, and it may be questioned whether the lymphocytes ever occur in true pus. Even in cases of lymphatic leukemia the predominating cell in abscesses is the polynuclear leukocyte or its degeneration forms. Mononuclear elements with basophilic protoplasm, however, are also met with, notably in the more chronic cases, but it is likely that they are derived from the connective-tissue cells and are not of hematogenic origin. Eosinophiles are only seen

in pus under certain definite conditions, as in gonorrhea (see below), and mast cells also are quite uncommon.

In pus that is not perfectly fresh it is usually not possible to demonstrate the presence of neutrophilic granules. In such cells, moreover, we commonly meet with fragmentation of the nucleus, associated with marked pyknosis. This was first noted by Ehrlich in a case of hemorrhagic smallpox and in various exudates, and has subsequently been described by Michaelis and Wolff. The degeneration may proceed to fragmentation of the entire cell, with the consequent formation of mononuclear neutrophilic forms (Ehrlich's pseudolymphocytes). On the other hand, a type of degeneration is seen in which the nucleus does not become pyknotic, but swells to a large size and stains rather faintly with basic dyes. In such cells the protoplasm appears as a narrow rim and the impression is gained as though the cell were in reality a leukocyte; if at the same time the granules have been lost, the differentiation may indeed be impossible, unless transition forms exist between the normal polynuclear neutrophile and the type in question.

Owing to resorption of water from accumulations of pus of long standing, such material finally assumes a caseous aspect, and the leukocytes will be seen to have greatly diminished in size, and to have assumed an angular, shrunken appearance; it is then hardly possible to demonstrate the presence of a nucleus, even after the addition of acetic acid.

It is noteworthy that in cases of hepatic abscess referable to Amœba coli it is seldom possible to demonstrate any normal leukocytes, and it will be seen that under such conditions the pus consists almost altogether of granular and fatty detritus, while in liver abscesses due to other causes the leukocytes usually present a fairly normal appearance.

Mast cells are only exceptionally seen in pus.

Giant Corpuscles.—So-called giant pus corpuscles, measuring at times from 30 to 40 μ in diameter, have been observed in abscesses of the gum, hypopyon, and in the contents of suppurating ovarian cysts, but they do not appear to have any special significance. Upon careful examination these bodies will be seen to contain one oval nucleus, usually located excentrically within the cell, and from one to thirty or even forty pus corpuscles.

Detritus.—Fatty and albuminous detritus in variable amount may be observed in every specimen of pus, and increases with the length of time that it has been confined within the body. The same holds good for the presence of free nuclei.

Red Corpuscles.—Red blood corpuscles in variable numbers are usually seen in every specimen, their appearance depending upon the length of time that they have been confined. Pus corpuscles may at times contain a red corpuscle.

Pathogenic Vegetable Parasites.—Of the pathogenic organisms which are of special interest from a clinical standpoint may be mentioned the true pus organisms, notably the staphylococci and the Streptococcus pyogenes, the gonococcus, the meningococcus, the colon bacillus, proteus, the tubercle bacillus, Actinomyces hominis, the bacillus of glanders, the bacillus of anthrax, leprosy, tetanus, influenza, the pneumococcus, etc. The majority of these have already been described. A pathogenic leptothrix, named by Flexner the L. asteroides, has been found by Cozzolino in the pus of a retroperitoneal abscess.

A form of streptothrix has been isolated from the pus of certain cases of mycetoma, or Madura foot.

Vincent's fusiform bacilli and spirilla have been encountered in the pus of alveolar pyorrhea, in noma, hospital gangrene, gangrenous ulcer of the penis, in bronchiectasis, abscess of the leg, etc.

In the pus of abscesses in cases of systemic blastomyces infection the corresponding organism is found.

Protozoa.—With the exception of amœbæ, protozoa have only rarely been found. Künstler and Pitres observed numerous large spores with from ten to twenty crescentic corpuscles in pus taken from the pleural cavity of a man, which closely resembled the coccidia of mice. Litten observed cercomonads in the fluid withdrawn from a pleural cavity. Trichomonads have been found in empyema in connection with pulmonary gangrene.

In cases of amœbic liver abscess with perforation into the lung the corresponding organism may be demonstrated in the purulent expectoration (see Sputum). Kartulis and Flexner have demonstrated amœbæ in the pus from an abscess of the lower jaw.

In the pus from alveolar and dental pyorrhea amœbæ are invariably found and are regarded by some as the causative factor of the disease. The two types which are usually seen are pictured in Plate XV. Satisfactory pictures are obtained by emulsifying a drop of the purulent material with a drop of saline on a slide and staining the air-dried specimen with a 1 per cent. aqueous solution of methylene blue. In this manner the amœbæ not only are well brought out, but also the spirochetes and bacteria which are simultaneously present in enormous numbers. Following the local and especially the hypodermic use of emetin the amœbæ (temporarily at least) disappear from the pus.

In smears obtained from two cases of oriental boil (tropical ulcer, Delhi boil, Aleppo boil), Marzinowsky and Bargow, on the one hand, and Wright on the other, found little bodies, measuring from 1 to 4μ in diameter and apparently provided with a macronucleus and a micronucleus. They are inclined to look upon these as protozoa and as parasitic. Marzinowsky and Bragow name the organism Boöplasma orientale; Wright calls it the Helcosoma tropicum. According

to Christofers they are identical with the Leishmania-Donovani of tropical splenomegaly, which latter are known to occur in the skin ulcers of kala-azar.

Vermes.—Of these, the filaria and hydatids are rarely observed in this country. Bothriocephalus linguloides has been found in the pleural cavity of a Chinese patient.

Crystals.—As has been stated, crystals of cholesterin are frequently found in old pus and in exudates of long standing, but are rarely seen in recent exudates. They may be recognized by their characteristic form and their chemical reactions, as described in the chapter on the Feces. Triple phosphates, fatty acid crystals, and hematoidin are likewise frequently seen, the presence of the latter, of course, indicating a previous admixture of blood.

Gonorrheal Pus.—For a consideration of the cytology of gonorrheal pus the reader is referred to the section on gonorrhea. The characteristics and staining properties of the gonococcus are described in the bacteriological appendix.

PUTRID EXUDATES

Putrid exudates are observed following perforation of a gangrenous focus or of a gastric or intestinal ulcer into one of the body cavities. At other times they are encountered in cases of neoplasm, and at times even without apparent cause. The material obtained in such cases has a brown or brownish-green color, and emits an odor which in itself indicates the character of the exudate. Microscopically, cholesterin, hematoidin, and fatty acid crystals, as well as degenerating leukocytes, are found. In cases in which aspiration of a higher intercostal space reveals the presence of serous fluid, while putrid material is obtained at a lower point, the existence of a subphrenic abscess should be suspected. In such cases a pure culture of the Bacillus coli communis has been obtained. The reaction of putrid exudates is usually alkaline, but an acid reaction may be obtained in cases of perforation of a gastric ulcer; the Sarcina ventriculi and saccharomyces may then also be found.

CHYLOUS AND CHYLOID EXUDATES

Chylous and chyloid exudates have been repeatedly observed. They are most frequently met with in the abdominal cavity (one hundred and four times out of a total number of one hundred and fifty-five, which have thus far been reported), less commonly in the pleural cavity (forty-nine times), and only rarely in the pericardial sac (twice only) (1904). Among the causes which may lead to chylous ascites the following are recognized (in the order of their frequency): compression of the thoracic duct or the lymphatic vessels by glandular

enlargements, neoplasms, etc.; non-tuberculous peritonitis; occlusion of the left subclavian; excessive pressure, strain, cough; peritoneal carcinoma; filariasis; occlusion of the thoracic duct; occlusion of lymph vessels, external pressure; diseases of the liver, syphilis, primary disease of the lymph vessels, angioma, calculus of the receptaculum chyli, and Hodgkin's disease. Quincke believes that the two forms can be etiologically distinguished from one another by means of a microscopic examination, as the cloudy appearance in the chyloid form is usually referable to the presence of endothelial cells undergoing fatty degeneration. Later observations, however, have shown that the differentiation of the two forms cannot be made upon this basis, as the same anatomical lesion, such as carcinoma or tuberculosis, may at times give rise to the formation of a chylous exudate, at others to that of the chyloid form, and both, moreover, may co-exist. An instance of this kind is described by Wilson.

Senator claimed that the presence of more than traces of sugar is strongly suggestive of the chylous nature of the exudate; but only the presence of more than 0.2 per cent. is of significance. More important is the fact that in chylous fluid the melting point of the fat will depend upon the melting point of the fat which was taken in as food, while this is not the case in chyloid effusions. The amount of fat, moreover, which is present is influenced directly by the amount ingested in the first instance.

Occasionally one can get the distinct odor of the food which has been taken, in chylous exudates, while in the chyloid type this would hardly be expected.

Chylous exudates in their general appearance resemble milk, while chyloid fluid is more suggestive of pus. The turbidity in both cases is usually referable to the presence of innumerable fat globules, which are especially abundant in the chylous form. In chyloid exudates the origin of the fat from cellular elements is often apparent at once; but, as has been said, it is impossible to draw definite etiological conclusions from that difference. Some chyloid exudates contain no fat at all, and Lion has shown that the milky appearance in such cases is owing to the presence of a curious albuminous substance, belonging to the class of nucleo-albumins. Bernert, on the other hand, claims that the substance in question belongs to the globulins, and is closely associated with certain lecithins. A similar observation is recorded by Micheli and Mattirolo.

Edsall (cited by Wilson) reported an instance of non-fatty pleural effusion, the opacity of which was due to altered globulins.

Chemical analysis of a chylous exudate (pleural) from a case of Hodgkin's disease, which Campbell made in my laboratory, showed the following result:

Water	90.84	per cent.
Solids	9.15	" "
Mineral solids	0.76	" "
Organic solids	8.39	" "
Coagulable albumins	4.80	" "
Fats	3.00	" "
Sugar	0.59	" "

The specific gravity was 1.020.

The cytological formula in such exudates has as yet received but little attention. In Campbell's case only a small number of leukocytes was present, and most of these were of the lymphocytic type. In Müttermilch's case lymphocytes were said to preponderate; in addition there were small numbers of neutrophilic leukocytes, containing fat granules, together with eosinophilic cells and a very few red cells. In the mixed case of Wilson the lymphocytes numbered 76 per cent., and the large mononuclear cells 22 per cent.

EXAMINATION OF SYPHILITIC MATERIAL

Spirochæte Pallida.—Through the researches of Schaudinn and Hoffmann it has been ascertained that in primary and secondary syphilitic lesions a spirochete can be demonstrated which probably represents the cause of the disease. Their results have been abundantly verified both abroad and in the United States. The organism has been demonstrated in the scrapings obtained from chancres, incised papules, and condylomata, and in smears from mucous patches and the aspirated juice of the inguinal glands. Schaudinn and Hoffmann could further demonstrate the organism in the blood obtained by puncture of the spleen in a recent case of syphilis on the day preceding the eruption. Levaditi found it in the vesicular contents of Pemphigus syphiliticus. Buschke and Fischer, Babes and Panea, and Levaditi found the spirochete in the internal organs of children who had died of congenital syphilis, as also in the blood, and Metchnikoff could demonstrate it in the lesions of artificial syphilis in the ape.

The Spirochæte pallida derives its name from its low refractive power and the difficulty with which it takes up anilin dyes (this especially in contradistinction to the Spirochæte refringens). It is a very delicate structure, usually presenting 10 to 40 deep spiral incurvations in the larger specimens, or only 2 to 4 in the smaller ones. The length varies from 4 to 10μ, with 7μ as an average; the width does not exceed 0.5μ. In the wet preparation it may be observed that its movements occur in an oscillatory manner about the longitudinal axis, and that, in contradistinction to the spirilla, the movements of the spirochete are winding, bending, and whipping, while in the spirilla the longitudinal axis remains rigid. Schaudinn also

PLATE XXXII

demonstrated the existence of a flagellum at each end, while the other spirochetes have an undulating membrane. (See Plate XXVII and Fig. 186.)

Demonstration of the Spirochetes.—The best results are obtained by examining the living organism by dark field illumination, using a drop of the serous fluid from the syphilitic lesion, mounted on a slide and covered with a cover-glass. An excellent apparatus for this purpose is furnished by E. Leitz & Co. In its absence the same effect may be obtained by mixing a drop of the serum with a drop of sterile India ink and examining this either directly in the wet state, covered with a cover-glass, or spread out like a blood smear, placing a drop of immersion oil directly upon the dried specimen.

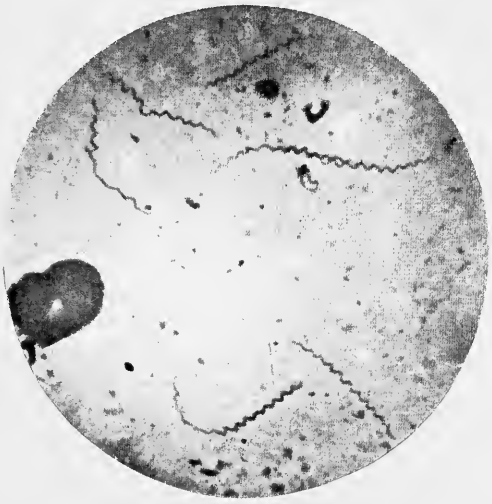


Fig. 186.—Spirochæte pallida.

Staining Methods.—Excellent results are obtained with Goldhorn's stain (which see). To this end the smears, on slides or covers, are covered with the dye for three or four seconds, when the excess is drained off. The specimens are then introduced *slowly* into clean water with the film side down, permitting in this manner an interaction between the film of adhering dye and the water. The slide is held in this slanting position for another four or five seconds and is next shaken in the water so as to wash off the excess of the dye. The pallida appears of a violet color, which may be changed to bluish black by flooding the preparation for fifteen to twenty seconds with Gram's iodine solution, washing and drying as usual. The examination is conducted with a $\frac{1}{12}$ or $\frac{1}{16}$ immersion lens. Giemsa's method also furnishes excellent results.

The material should in all cases be obtained by curettage, this

being carried so far until a small amount of serum and blood appears, and preferably at the edge of the lesion. The serous fluid is then spread upon slides or covers in the usual manner. The organisms are most numerous in moist papules and chancres (when the curettage is carried out at the edge of the lesion). In roseolar scrapings the search is frequently disappointing.

To isolate the organism for purposes of culture, Noguchi recommends the following procedure: Material from the syphilitic lesion is inoculated into tubes containing serum water and small pieces of fresh sterile tissue. The tubes measure about 1.5 cm. in diameter and 20 cm. in length. The serum water (1:3 of distilled water) is best prepared from human serum (ascitic fluid, pleuritic effusions, cystic fluid), but in its absence sheep serum or rabbit serum may also be employed. Each tube receives about 16 c.c. of the diluted serum. The tubes are sterilized by steaming for three consecutive days for fifteen minutes at a time, when a bit of fresh sterile tissue (rabbit kidney, testicle, heart muscle, sheep kidney, testicle—no liver), measuring about 0.5 to 1 cm. square is added to each, and the surface is covered with about 3 c.c. of sterile paraffine oil. The tubes must of course first be incubated to prove their sterility. After inoculation with the syphilitic material they are then placed in a desiccator, containing pyrogallic acid, and kept under strictly anaërobic conditions (atmosphere of hydrogen; exhaustion of air with vacuum pump) at a temperature of from 35° to 37° C. Growth commences after forty-eight hours and continues for four or five weeks or longer.

While the anaërobic conditions of course interfere with the growth of some of the contaminating bacteria, the primary cultures will be found to be infected, and the difficulty now is to obtain the spirochete by itself. To this end a small amount of the cultures in which spirochetes have been demonstrated by examination in the dark field is inoculated by a stab into ascitic serum agar containing a little tissue (as above), when the bacteria will grow only along the line of the stab while the spirochete enters the substance of the agar (in the neighborhood of the piece of tissue), where they give rise to a slight haziness, which becomes perceptible after two to four days, but frequently does not become distinct until a week or ten days have elapsed. By cutting through the tube about this point with a diamond pencil, the culture of the spirochete can now be approached from the outside, where there is less chance of contamination from the associated bacteria, when subcultures may be prepared in serum agar or serum water, in the presence of tissue. It should be pointed out, however, that pure cultures can be obtained only with much difficulty and only too often the organism may be lost altogether.

CHAPTER IX

THE CEREBROSPINAL FLUID

GENERAL CHARACTERISTICS OF CEREBROSPINAL FLUID

WITHIN recent years puncture of the vertebral canal has been frequently resorted to for diagnostic purposes. The practical value of this method of diagnosis is now beyond question, and it is to be hoped that ere long physicians will resort to spinal puncture in obscure cases of cerebrospinal disease with as little hesitancy as puncture of the thoracic and abdominal cavities is now practised.

The *operative method* to be employed is the following: With the patient placed upon his left side—some observers prefer the sitting posture—and the body bent well forward, a long aspirating needle is introduced upon a level with the lower third of the third or fourth lumbar spinous process, and about 1 cm. to the side of the median line, the needle being directed slightly upward and inward. The depth to which it is necessary to puncture will, of course, vary with the age of the patient. In a child two years of age the vertebral canal may be reached at a depth of 2 cm., while in the adult it is necessary to insert the needle for a distance of from 4 to 8 cm. As soon as the subarachnoid space is reached cerebrospinal fluid will flow from the needle. *Aspiration* should always be avoided.

Some writers have advised that the operation be performed under narcosis; and without doubt this may be necessary at times, particularly when contracture of the dorsal muscles exist. In the majority of cases, however, it is not necessary, and local anesthesia will suffice.

Amount.—So far as I have been able to ascertain, no observations have been made regarding the amount of fluid which may be obtained by puncture in normal individuals. In all probability, however, this is small. Under pathological conditions the amount may vary from a few drops to 100 c.c., and even more. In general terms it may be stated that the amount is directly proportionate to the degree of intracranial pressure. Exceptions, however, are frequent. Small amounts of cerebrospinal fluid or none at all may thus be obtained, when, owing to the formation of a thick exudate or the existence of a cerebral tumor, communication between the basilar subarachnoid spaces of the brain and those of the spinal cord has been interrupted. Whenever, then, symptoms of intracranial pressure exist while no fluid or minimal amounts only can be obtained by puncture, the

conclusion will usually be justifiable that we are dealing with a purulent meningitis or with a tumor of the brain, and more especially of the cerebellum. It should be remembered, however, that the same result may be obtained in cases of obliteration of the aqueduct of Sylvius, or when sclerotic processes involve the foramen of Magendie, which is occasionally observed in certain forms of hydrocephalus. Adhesions of the pia mater to the arachnoid and the dura mater may, by interfering with the flow of cerebrospinal fluid, also lead to the formation of hydrocephalus, but in these cases a tumor can usually be excluded, as the changes in question always develop as sequels to a meningitis. A serous or tuberculous meningitis, as well as acute hydrocephalus and tetanus, can, however, always be excluded when only minimal amounts of fluid are obtained by puncture. The largest amounts, on the other hand, are seen in cases of serous meningitis, tubercular meningitis, and cerebral tumors, which do not interfere with the circulation of the cerebrospinal fluid. In the epidemic type of meningitis 70 to 80 c.c. can usually be obtained very readily. In epilepsy Pellagrini usually obtained amounts varying between 10 and 15 c.c. Donath gives rather higher figures, up to 60 c.c., and in a tabes case, 85 c.c.

Appearance.—Normal cerebrospinal fluid, as well as that obtained in cases of serous meningitis, tuberculous meningitis, hydrocephalus, and tumors of the brain, is perfectly clear, and, as a rule, colorless unless a small bloodvessel has been punctured, when the fluid may present a slightly reddish tinge. More or less pronounced yellow shades are, however, at times observed. Important from the standpoint of diagnosis is the fact that in cases of hemorrhage into the ventricles pure blood is found. (See Cerebral Hemorrhage.)

Cloudy fluid is obtained in all cases of purulent meningitis unless the disease is limited to a very small area. In the epidemic type, however, it may be quite clear, or but slightly cloudy. Cases of abscess of the brain or sinus thrombosis occur again and again in which the question as to the advisability of operative interference is largely dependent upon the presence or absence of a complicating purulent meningitis. In certain instances a satisfactory conclusion may, of course, be reached without puncture; but in many others this is impossible, and Lichtheim's dictum, that an operation should never be undertaken in such cases unless the integrity of the meninges has been established by spinal puncture should be borne in mind.

The degree of cloudiness naturally varies in different cases, and while in some instances the character of the fluid is seropurulent, pure, creamy pus may be found in others. Generally speaking, a cloudy fluid indicates the existence of an acute inflammatory process or an exacerbation of a chronic process.

Important, furthermore, is the fact that the fluid in non-inflammatory diseases of the brain, such as tumor or abscess, rarely under-

goes coagulation, while this is the rule in all inflammatory diseases. In tubercular meningitis the coagula are very delicate, and may be well compared with spider-webs extending throughout the fluid, while in purulent meningitis the coagula are somewhat firmer.

Specific Gravity.—The specific gravity of cerebrospinal fluid normally varies between 1.005 and 1.007, corresponding to the presence of from 10 to 15 pro mille of solids. Under pathological conditions, variations from 1.003 to 1.012 may be observed, the specific gravity, generally speaking, being higher in the inflammatory than in the non-inflammatory diseases of the brain. From a diagnostic standpoint, however, the determination of the specific gravity is of little value, as numerous exceptions occur to the above rule.

Reaction.—The reaction is always alkaline.

CHEMICAL COMPOSITION OF CEREBROSPINAL FLUID

An idea of the chemical composition of the cerebrospinal fluid may be formed from the following analyses, taken from Gautier and Zdarek:

	Per cent.
Water	987.00
Albumin	1.10
Fat	0.09
Cholesterin	0.21
Alcoholic and aqueous extract, minus salts } Sodium lactate }	2.75
Chlorides	6.14
Earthy phosphates	0.10
Sulphates	0.20

ZDAREK'S ANALYSIS

Water	989.54
Solids	10.45
Organic solids	2.09
Mineral ash	8.35
Albumins	0.76
Ethereal residue	0.35
Aqueous residue	8.22
Sulphuric acid (SO_3)	0.04
Chlorin	4.24
Carbon dioxide	0.49
Potassium oxide	0.16
Sodium oxide	4.29
Mineral ash, insoluble in water	0.16
Glucose	0.10

Urea.—In addition, urea is at times found, as also a substance which reduces Fehling's solution and gives rise to a brown color when boiled with caustic potash, but which neither undergoes fermentation nor forms an osazone when treated with phenylhydrazin. The substance in question is generally regarded as pyrocatechin. Its amount varies between 0.002 and 0.116 per cent. According to C. Bernard, glucose

may also be present, but it is questionable whether this is the case under normal conditions (see below). Nawratzki discovered a reducing substance in his cases, which was demonstrated to be glucose; his subjects, however, were unfortunately not normal, but general paretics with fever. Pyrocatechin was absent. Zdarek reports a recent case of anterior meningocele in an otherwise normal individual in which the fluid reduced Fehling's solution and gave a glucosazone with phenylhydrazin. The substance in question was dextrorotary, the amount equalling 0.1 per cent. of glucose.

Glucose.—Lichtheim claims to have found glucose—by means of the phenylhydrazin test—in all cases of tumor which he examined. In cases of tubercular meningitis, on the other hand, a positive result was only exceptionally obtained. Quincke also reports that he was able to demonstrate the presence of sugar whenever the liquid obtained was sufficient in amount for the necessary tests. Unfortunately, however, he does not detail his cases. Concetti found no sugar in hydrocephalic fluid.

The experience of other observers does not agree with that of Lichtheim and Quincke; and Fürbringer, who has thus far reported the largest number of spinal punctures, found sugar in only 2 cases of diabetes associated with tuberculosis.

Cholin.—Cholin, contrary to former views, has now been shown not to occur in the cerebrospinal fluid (Kauffmann).

Albuminous Bodies.—So far as the albuminous bodies are concerned which may be found in the cerebrospinal fluid, serum albumin is said to be present only under exceptional conditions, while normally a mixture of globulin and albumoses is found. The question whether or not mucin may also be present is still undecided.

Under pathological conditions the amount of albumin may vary considerably, and is of diagnostic importance. The lowest values have been obtained in cases of chronic hydrocephalus (traces only), meningitis serosa (0.5 to 0.75 pro mille), and tumors of the brain (traces to 0.8 pro mille); while the largest amounts have been found in chronic hydrocephalus the result of hyperemia (1 to 7 pro mille), and in tubercular meningitis (1 to 3 pro mille). Nawratzki in recent examinations found amounts varying between 0.047 and 0.170 per cent., but the subjects of his investigation had fever at the time. Mott and Halliburton found three times the normal amount of albumin in paralytics, as also some nucleo-albumin, which does not occur in health. The latter they supposed to have come from broken-down Nissl bodies.

Noguchi's Butyric Acid Test.—This test is based upon the observation of Noguchi that in syphilitic and parasyphilitic affections the globulin content of the blood serum and cerebrospinal fluid is inincreased, and that this increase is more constant and more marked than the content in the "Wassermann antibody." It is applicable

particularly in the examination of the cerebrospinal fluid, but may be used also, in a modified form, in the case of the blood serum.

Test as Applied to the Cerebrospinal Fluid.—0 1 or 0.2 c.c. of the meningeal fluid, which must be entirely free from blood, is treated with 0.5 c.c. of a 10 per cent. solution of butyric acid in normal saline, and boiled for a few moments over a flame, when 0.1 c.c. of normal sodium hydrate solution is quickly added and the mixture is boiled for a few seconds longer. In the presence of an increased protein content a granular or flocculent precipitate develops, which gradually settles to the bottom of the tube. The rapidity with which the reaction occurs, and the volume of the precipitate will depend, of course, upon the amount of protein present. When this is large, the precipitate appears in granular form, within a few minutes, while two hours may elapse if the increase is only slight. This, indeed, Noguchi indicates as the time limit.

Test as Applied to the Blood Serum.—0.5 c.c. of clear serum, which must be free from hemoglobin, is treated with 4.5 c.c. of a half saturated solution of ammonium sulphate and centrifugalized at high speed (5000 revolutions per minute) for at least thirty minutes. The supernatant fluid is carefully pipetted off and the precipitate dissolved in 5 c.c. of 0.9 per cent. salt solution. Of the resultant solution 0.5 c.c. is mixed with an equal volume of the butyric acid solution (see above). If the blood was from a syphilitic patient a dense, milky turbidity appears at once, while with normal serum the solution remains clear or shows a slight opalescence only, even after standing for two hours.

Noguchi recommends that the examination of a number of specimens be carried out at the same time and that a normal serum should always be included in the series.

Nonne or Ross-Jones Reaction.—Equally satisfactory results may be obtained by superimposing the cerebrospinal fluid upon a neutral saturated solution of ammonium sulphate, as suggested by Nonne and Ross-Jones, when a distinct white ring will appear within a minute or two if globulins are present in abnormally large amount. This constitutes the so-called Phase I of Nonne. For routine purposes it is best to use 1 c.c. of the reagent and 1 c.c. of the fluid and to read the results after three minutes. Blood must of course be absent. The reaction is supposedly due to the presence of globulins and nucleoproteins.

Pandy's test is based upon the same principle and is possibly more sensitive even than the Ross-Jones test. The reagent is a saturated aqueous solution of carbolic acid. One drop of spinal fluid is added to form 0.5 to 1.0 c.c. of the reagent. Thus in the presence of an abnormal protein content a bluish-white ring or cloud forms immediately. The admixture of blood must of course be carefully avoided.

(For a discussion of the results which may be obtained in the different types of syphilis, see the section on Syphilis.)

The Lange Colloidal Gold Reaction.—Following the discovery by Zsigmondy that proteins can prevent the flaking out of colloidal gold (goldsol) on the part of electrolytes, and that the "protective" power of the proteins in this direction varies with different bodies, but is constant for one and the same, Lange sought to utilize this principle in the study of blood sera and cerebrospinal fluid, and more particularly in the attempt to distinguish between syphilitic and non-syphilitic material. In the course of his experiments he made the *incidental* observation that sera when diluted with 0.4 per cent. saline to a certain degree will flake out goldsol by themselves, while the same sera when diluted with distilled water did not possess this property. On first consideration, one might be tempted to ascribe the flaking out in the first instance to the 0.4 per cent. saline, but as a matter of fact the quantity of this reagent which is actually present in the experimental tube does not possess this power. On the other hand, it was shown that after coagulating the proteins by various methods the goldsol was flaked out nevertheless, and that luetic sera did this more markedly than non-luetic specimens. The results, however, were not sufficiently satisfactory as to warrant the application of this method of examination to the diagnosis of syphilis, which Lange originally had in mind. On applying the observations just made to the study of cerebrospinal fluid, however, he obtained more satisfactory results. Normal fluid, when diluted with 0.4 saline, gave rise to no separation of the gold, while under certain pathological conditions this occurred, and it was ascertained, moreover, that the type of response was in a certain measure characteristic of certain maladies, and among these of syphilis, affecting the central nervous system. These observations have in the main been confirmed, and Lange's reaction is now recognized as one of the standard methods employed in the diagnosis of pathological processes involving the cerebrospinal axis.

Technique.—*Apparatus.*—1. A couple of Jena beakers of 1500 to 2000 c.c. capacity (preferably the tall form).

2. A few 1 c.c. pipettes, graduated in hundredths.
3. A few 10 c.c. pipettes, graduated in ones.
4. Test tubes (20 c.c. capacity).
5. Test-tube rack holding 12 or 13 tubes.
6. A Liebig distilling apparatus.
7. Jena flasks (2000 c.c. capacity) for distilling water.

All Glassware Must be Chemically Clean.—To this end it is first cleansed with a mixture of concentrated commercial sulphuric acid, and a strong solution of potassium bichromate, then with tap water in abundance, and finally with doubly distilled water.

Reagents.—1. Distilled water. *All reagents must be prepared with absolutely pure distilled water,* and to this end it is urgently recommended to use *only doubly* distilled water, and to test this with

a little gold chloride before making up larger amounts. Rubber stoppers should be avoided in connection with the distilling apparatus as well as with the reagent bottle; glass stoppers are the best, while cork may be used with the still.

2. A 1 per cent. solution of chloride of gold (Aurum crystallisatum flavum, Merck) in doubly distilled water.

3. A 2 per cent. solution of potassium carbonate (purissimum) in doubly distilled water.

4. A 1 per cent. solution of formalin in doubly distilled water.

5. From these reagents the goldsol is prepared as follows: 100 c.c. of doubly distilled water are treated with 1 c.c. of the goldsol and 1 c.c. of the carbonate solution. The liquid is heated to boiling (until the finer size of bubbles which form throughout the fluid, in contradistinction to the large ones which come from the bottom of the beaker, disappear), when 1 c.c. of the formalin is added. If reagents and glassware have been faultless the resultant solution assumes a deep purplish-red tone without any tinge of blue. Under these conditions it is then warrantable to proceed with the preparation of a larger quantity, *e. g.*, 1000 c.c., using the reagents in the same proportion.

Miller and his collaborators have pointed out that the final product must be neutral, as otherwise non-typical or non-specific reactions are obtained. When first made, the colloidal gold solution is apt to be acid while with time it tends to become alkaline. It is suggested to neutralize the solution only, after it is at least forty-eight hours old, and to examine it from time to time, as it is apt to become alkaline again even after neutralization. In any event a neutral solution only should be employed for the test. For purposes of neutralization a $\frac{N}{50}$ solution of sodium hydrate is used, with alizarin red (a 1 per cent. solution in 50 per cent. alcohol) as indicator, the neutral point being indicated by a brownish-red color.

The cerebrospinal fluid must be free from blood; unless this is assured the results will be valueless. To this end it is advisable to collect the fluid in several portions, and to use only the last few drops for the reaction. If blood is macroscopically visible in the first portion, it is better not to resort to this method of examination at all.

The needles which are used for puncture should not be allowed to become rusty. After use, they should be cleansed with saline, then with alcohol and finally with ether; they may then be placed in test tubes in cotton, and sterilized by dry heat; then they are ready for use. If the pressure of the cerebrospinal fluid is to be determined at the time of its collection, care should be had to use short rubber connections only, and to watch these carefully, so that no admixture of particles of rubber can occur.

Method.—The fluid under investigation is diluted 1 in 10 with 0.4 per cent. of saline (prepared with doubly distilled water). A series of

tubes is then charged with progressive dilutions of this stock dilution. To this end it is convenient to proceed as follows:

Tube 1 receives 1 c.c. of the stock dilution (1 in 10); the other tubes are charged with 1 c.c. of saline. Tube 2 receives in addition 1 c.c. of the stock dilution, making 2 c.c. of a 1 in 20 dilution. 1 c.c. of this is transferred to tube 3, a 1 in 40 resulting, from which 1 c.c. is in turn transferred to tube 4, giving rise to a 1 in 80 dilution, and so on through the series, which should be continued to approximately 1 in 40,000, making 13 tubes in all. Care should of course be had that the saline and the diluted fluid are always intimately mixed. 5 c.c. of the gold solution are then added to each tube, and the contents well mixed. The results are noted after several hours' standing (the following morning). Artificial light does not permit the recognition of changes to bluish red.

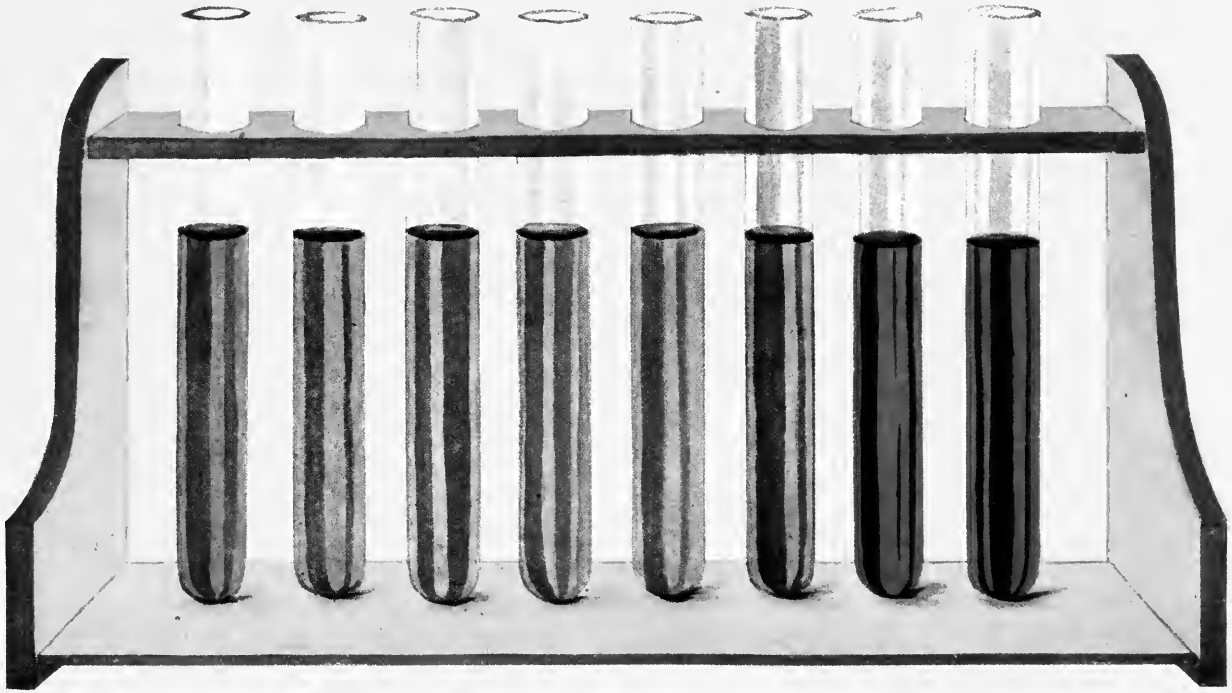
With normal cerebrospinal fluid it will be noted that even after twenty-four hours the gold solution remains unchanged, in every dilution from 1 in 40 to 1 in 40,000. In the case of pathological fluids, on the other hand, a flaking out of the gold may occur which is indicated by a change in color tending to a bluish tone through reddish blue, bluish red, lilac, to dark blue, and in the case of higher grades of flaking out from dark blue to light blue, and finally to colorless, a bluish-black sediment forming at the bottom of the tube varying in extent with the intensity of the reaction, *i. e.*, with the degree to which the flaking out process has taken place. At the same time it will be noted that the maximal effect is not obtained with the higher concentrations of the fluid. Instead of this, it will be observed that in a given case there is an optimal dilution at which the maximal flaking out occurs, and that beyond this point the process is less marked or does not occur at all. A reaction in which complete decolorization occurs, may be designated as a +++ reaction (see Plate XXVIII), while one in which a mere change from deep purple red to bluish red takes place may be reported as ± or doubtful. The question of course arises whether to regard such feeble reactions as diagnostically of significance, or whether they may not be due to accidental factors, such as the use of test tubes that were not perfectly clean, etc. When due to such a factor it will usually be found that it is only a single tube that is off color, while in a "proper reaction" there is a gradual transition to this point.

The results can be graphically represented in a curve, the degrees of dilution being noted on the abscissa, and the shades of color on the coördinate, as shown in the accompanying example (Fig. 187).

Instead of charting the result in the form of a curve, Miller has suggested a numerical expression which is based upon the designation of a given color by a given number as shown in the accompanying example (Fig. 188).

The most interesting factor in connection with Lange's reaction

PLATE XXVIII



Lange Colloidal Gold Reaction.

(Case of Paresis.)

is the fact that curves of different form are found in different types of diseases affecting the cerebrospinal axis, and that these curves appear to be constant for the maladies in question.

In syphilis involving the central nervous system the maximal flaking occurs in the dilution $\frac{1}{40}$ or $\frac{1}{80}$, and occasionally in maximal reactions it may extend to $\frac{1}{160}$; but in such cases 1 in 10 already will show a bluish shade.

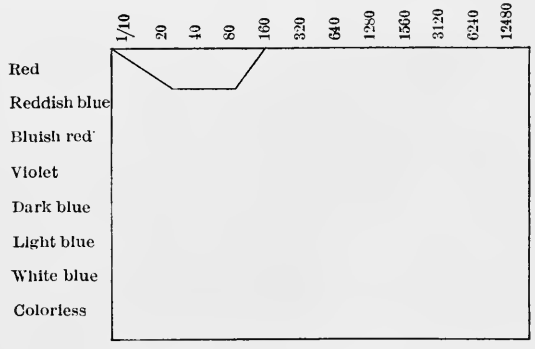


FIG. 187.—Case of secondary lues with headaches.

In non-syphilitic tumors the maximal point is farther to the right (Fig. 190). But the most marked deviation from the typical lues curve is seen in certain cases of purulent meningitis when flaking out only occurs in high dilutions (Fig. 189). This, however, does not appear to be constant.

So far as the general frequency is concerned with which a positive reaction occurs in syphilis, Lange has pointed out that this is by no means to be expected in all cases, but that involvement of the cerebrospinal tract on the basis of the colloidal gold reaction is of much more frequent occurrence than the ordinary clinical methods of examination would suggest.

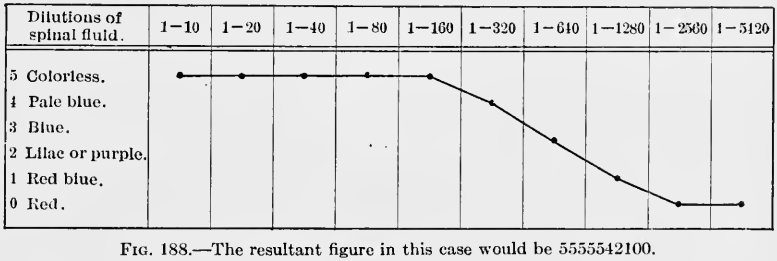


FIG. 188.—The resultant figure in this case would be 5555542100.

Lange's reaction has now been tested by many observers, and it seems to be the consensus of opinion that it is of definite value in the diagnosis of syphilis involving the central nervous system. It seems to be more delicate than either the blood or spinal Wassermann, the

cell count or the globulin reaction, but should invariably be controlled by all of them, and interpreted in connection with the clinical findings. When doubtful results are obtained careful reëxaminations are of course imperative.

In a study of the reaction from the psychiatric clinic of the Johns Hopkins Hospital, Miller, Brush, Hammers and Felton conclude as follows regarding the occurrence of the reaction in paresis: "The reaction type observed in cases of paresis has been so uniformly present and so characteristic as to warrant the following conclusions: (*a*) Spinal fluids from *clinical* cases of dementia paralytica cause complete precipitation of the colloidal gold in the first 4 to 8 tubes. Exceptions to this rule have not been observed. (*b*) The apparent specificity of the paretic reaction is further shown by its occurrence in a number of typical cases in which all other spinal fluid abnormalities were absent. One such case was confirmed by autopsy. (*c*) The fact that a paretic curve *occasionally* occurs in patients who show no evidence of dementia, in no way argues against the value of

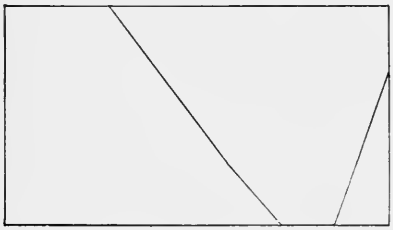


Fig. 189.—Purulent meningitis.

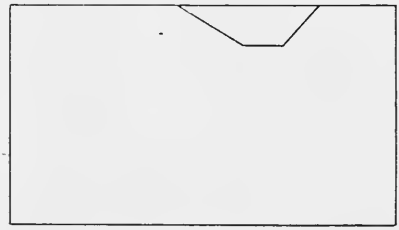


Fig. 190.—Brain tumor.

the reaction. All such reactions have occurred in cases undoubtedly luetic with the exception of a few of multiple sclerosis; none of these have been followed long enough to make certain that paresis will not ultimately develop. One must not loose sight of the fact that authentic cases are on record in which without the existence of any obvious psychosis general paralysis was discovered at autopsy. (*d*) The opinion is therefore advanced that the occurrence of a paretic reaction in a luetic individual should invariably be looked upon as one of grave portent.

In suspected cases of congenital syphilis, the writers did not obtain sufficiently characteristic reactions as to warrant a positive diagnosis.

So far as the general reactions go which are observed in tabes and cerebrospinal syphilis, these are not in themselves characteristic of either condition, and merely typical of the luetic type.

SEROLOGICAL EXAMINATION

The Wassermann Reaction.—In all cases of suspected syphilis of the central nervous system the Wassermann test should be applied

to the cerebrospinal fluid, even though the blood test has given a negative result. The technique is the same as when applied to the blood serum, though it is advisable to work with undiluted as well as with diluted fluid. As our standard volume for all the reagents employed is 0.5 c.c., no matter what the dilution may be, one can put up a series of tubes, containing increasing quantities of the cerebrospinal fluid, beginning with 0.1 c.c. up to 0.5 c.c., diluting with saline, whenever necessary up to 0.5 c.c. It will then be noted that some specimens only give a positive reaction, in the stronger concentrations, and these would have been erroneously looked upon as negative if the ordinary dilution of 1 in 5 only had been examined.

Non-syphilitic cerebrospinal fluid never furnishes a positive result when used in concentrated form.

PROTECTIVE FERMENTS

Protective ferments, in the sense of Abderhalden, have thus far not been found in the cerebrospinal fluid.

MICROSCOPIC EXAMINATION

Cytology.—Normal cerebrospinal fluid contains either no morphological elements at all or only a small number of lymphocytes (three to eight to the c.mm.). Deviations from this normal condition, as has been first shown by Widal, Ravaut, and Sicard, may be of marked diagnostic value.

While an actual increase in the total number of the cells, of course, warrants the conclusion that some factor is operative which deleteriously affects the cerebrospinal axis, it does not permit any conclusions regarding the nature of the pathogenic agent which is at work. Conditions here are exactly comparable to what we have found to hold good in the blood, viz., that the relative count is of far greater value than the absolute count. Here as there a polynucleosis is indicative of the existence of a pyogenic infection, while a lymphocytosis is noted especially in connection with tubercular and syphilitic lesions, and hence in paresis, tabes and cerebrospinal syphilis; it is common, moreover, in trypanosome infections (African sleeping sickness), in saturnine encephalopathy, and in anterior poliomyelitis. In the latter condition the neutrophile values are lower than 25 per cent. in nearly 90 per cent. of all cases. (See Part II.)

Among the syphilitic affections involving the central nervous system the highest counts are seen in paresis, where 300 cells have been noted per c.mm. More usual figures are from 20 to 60 and at times normal values may be observed (at least temporarily, or as the result of intraspinous treatment). In tabes the values average a little lower than in paresis, while in cerebrospinal lues the findings are more inconstant. (See Part II.)

In the epidemic form of cerebrospinal meningitis the predominating cell is the polynuclear neutrophile, excepting in chronic cases, where lymphocytes may prevail. This cell also enters into the foreground as recovery occurs.

Donath summarizes his results in 98 cases as follows: In acute and purulent meningitis polynuclear leukocytes prevail; in chronic or less intense processes, especially in tubercular meningitis, lymphocytes predominate. In the differential diagnosis of syphilitic meningitis, the early stages of tabes and of general paresis, from neurotic conditions and other malignant processes, lymphocytosis points to the first group. In tetanus a large number of polynuclear neutrophiles may also occur.

While in cerebrospinal meningitis referable to the Diplococcus pneumoniæ polynuclear leukocytosis is probably the rule, exceptions occur. Goggia thus reports a fatal case in which daily examinations showed a predominance of the small mononuclear elements throughout the course of the disease.

In connection with cerebral hemorrhage (especially hemorrhage into the ventricles) Sabrazès and Muratet have described the occurrence of large, round, oval, or polyhedral cells, either singly or in plaques, provided each with a single oval nucleus containing several nucleoli. These cells commonly contain red blood corpuscles, often in large numbers, as also crystals and amorphous particles of hematoidin, leukocytic nuclear debris and vacuoles. These cells are macrophages, derived undoubtedly from the endothelial lining of the subarachnoid spaces. Besides, granular structures may be met with which may contain globules of fat, nuclear debris, globules of myelin, red cells, and blood pigment. What these latter cells are is not known. Sabrazès inclines to view them as neuroglia cells.

Technique.—*Total Count.*—Specimens containing blood are not suitable for the cell count; for this reason it is best to use only the last portion of the fluid which flows through the needle. As a general rule satisfactory results may be obtained by counting the cells in the hemocytometer after having mounted a specimen from the native fluid direct. Beginners, however, frequently mistake red cells for lymphocytes, and to avoid error from this source it is recommended to mix equal parts of the fluid and of a very dilute solution of methyl violet in saline.

For differential counting smears are prepared from the centrifugate and best stained with plain aqueous methylene blue (1 per cent.). The polychrome dyes are not satisfactory, as the specimens usually become overstained.

Bacteriology.—Very important from a diagnostic standpoint is the fact that pathogenic microörganisms may be found. Lichtheim, Fürbringer, Freyhan, Dennig, Fränkel and many others since, were thus able to demonstrate the presence of *tubercle bacilli* in a fairly

large number of cases of tubercular meningitis (which see). In order to examine for tubercle bacilli, the fluid should be placed on ice for from six to twenty-four hours, until a slight coagulum has formed, when the fine, spider-web-like threads of fibrin are transferred to a cover-slip, spread in as thin a layer as possible, and stained as described in the chapter on the Sputum. If a centrifugal machine is available, the examination may, of course, be made at once; the chances of finding the bacilli are then also much greater. In every case a large number of specimens should be prepared before the search is abandoned. Only a positive result, however, is of value, and in doubtful cases recourse should be had to the animal experiment.

In the diagnosis of epidemic cerebrospinal meningitis lumbar puncture is of signal value, as the *Diplococcus meningitidis intracellularis* (meningococcus) of Weichselbaum- Jäger can be demonstrated in a large percentage of cases. (See Epidemic Meningitis.)

Mixed infections are not uncommon in epidemic cerebrospinal meningitis. Councilman thus found the pneumococcus in 7 cases and Friedländer's bacillus in 1. Terminal infections with staphylococci and streptococci also occur.

In other forms of purulent meningitis a large variety of organisms has been found. Wolf gives the following figures, resulting from an analysis of 174 cases, in which epidemic cerebrospinal meningitis is, however, included: in 44.23 per cent. the pneumococcus was found; in 34.48 per cent. the Diplococcus meningitidis intracellularis; in 3.45 per cent. staphylococci; in 8.03 per cent. streptococci; in 1.13 per cent. the bacillus of Friedländer; in 2.87 per cent. the Bacillus typhosus; in 1.72 per cent. the bacillus of Neumann-Schäffer, and in 2.87 per cent. the Bacillus coli communis, the Bacillus pyogenes fœtidus, the Bacillus aërogenes meningitidis, and the Bacillus mallei; while no bacteria were found in 1.15 per cent. of the cases. In 2 cases Pfeiffer's influenza bacillus has also been encountered in the cerebrospinal fluid during life.

In the African sleeping sickness *trypanosomes* are commonly found in the cerebrospinal fluid, obtained by lumbar puncture. Castellani obtained the organism in 20 cases of 34, and Bruce found it in all of 38 cases. (See Blood.). The results of these earlier observers have been abundantly confirmed. In many cases, however, the parasites never find their way into the cerebrospinal fluid. They are more frequently found toward the termination of the disease. Large numbers are rare; if they do occur there is usually an access of temperature. When present, the leukocytes are apt to be increased. There is no relation between the number present in the blood and in the spinal fluid.

CHAPTER X

THE EXAMINATION OF CYSTIC CONTENTS

CYSTS OF THE OVARIES AND THEIR APPENDAGES

The material obtained from cysts of the ovaries or their appendages varies greatly in character. On the one hand, it may be fluid, clear, of low specific gravity, and contain little albumin; while on the other it may be dense, viscid, and of colloid appearance. The specific gravity varies between 1.018 and 1.024, owing to the presence of a large amount of albumin.

In addition to smaller amounts of serum albumin and serum globulin the fluid of ovarian cysts contains a considerable quantity of another albuminous substance, which is termed *metalbumin* (Scherer) or *pseudomucin* (Hammarsten). Like Hammarsten's mucoid of transudates, it cannot be directly precipitated with acetic acid, but must be isolated as follows:

Test for Pseudomucin.—The fluid is mixed with three times its volume of alcohol and set aside for twenty-four hours, when it is filtered and the precipitate suspended in water. This is again filtered and the filtrate tested in the following manner: (1) A few cubic centimeters are boiled, when in the presence of metalbumin the liquid will become cloudy, without the formation of a precipitate. (2) With acetic acid no precipitate is obtained. (3) Upon the application of the acetic acid and potassium ferrocyanide test the liquid becomes thick and assumes a yellowish color. (4) When boiled with Millon's reagent a few cubic centimeters of the filtrate will yield a bluish-red color, while the addition of concentrated sulphuric acid, without boiling, gives rise to a violet color.

Paramucin is another albuminous substance which is found in colloid cysts and belongs to the mucinoid bodies. Like the true mucins and the body which occurs in exudates the paramucin is also precipitated by dilute acetic acid.

The color of cystic fluids may vary from a light straw to a reddish brown, or even a chocolate; the latter color may be observed when hemorrhage has taken place into the cyst.

Of morphological elements, ovarian cysts contain red blood corpuscles, leukocytes, and at times fatty granules in large numbers, crystals of cholesterin, hematoidin, and fatty acids. Most important, however, from a diagnostic standpoint is the presence of cylin-

drical or prismatic, ciliated epithelial cells, derived from the internal lining of the cyst, in the presence of which the diagnosis may be definitely made (Fig. 191). At times such cells cannot be demonstrated, as they may have undergone fatty degeneration; moreover, if the epithelium lining the cyst is squamous in character, it may be difficult, if not impossible, to arrive at a satisfactory conclusion from an examination of the morphological elements alone. *Colloid concretions*, which may vary in size from several micromillimeters to 0.1 mm., are occasionally observed, and more particularly in colloid cysts. They may be recognized by their irregular form, homogeneous appearance, slightly yellow color, and delicate outlines.

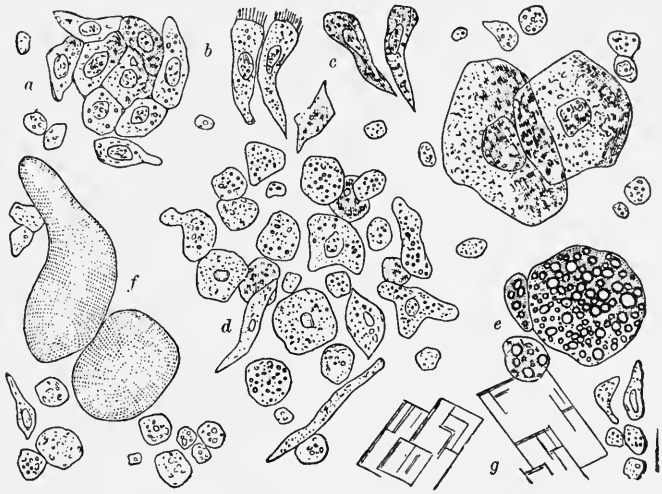


FIG. 191.—Contents of an ovarian cyst: *a*, squamous epithelial cells; *b*, ciliated epithelial cells; *c*, columnar epithelial cells; *d*, various forms of epithelial cells; *e*, fatty squamous epithelial cells *f*, colloid bodies, *g*, cholesterin crystals. (Eye-piece III, obj. 8 A, Reichert.) (v. Jaksch.)

In dermoid cysts, epidermal cells and occasionally hairs are observed.

The differential diagnosis of ovarian, parovarian, and fibrocystic (uterine) cysts cannot always be made from the character of the fluid withdrawn by puncture, but at times it is possible. The most important points of difference are here given: (1) The fluid in ovarian cystomas is usually more or less viscid, and often contains nonnucleated granular corpuscles of about the size of leukocytes, the granules of which do not dissolve in acetic acid nor disappear when treated with ether. In all probability they are free nuclei; in the United States they are often called Drysdale's corpuscles. (2) In parovarian cysts the fluid is thin, watery, of low specific gravity (under 1.010), and contains very few morphological elements. Cylin-

drical epithelium is very rarely found during life in the fluid withdrawn by aspiration from either ovarian or parovarian cysts. (3) The fluid from fibrocystic tumors of the uterus is thin, watery, and coagulates spontaneously, while that from ovarian and parovarian cysts never coagulates spontaneously unless blood is present. Fibrocystic tumors of the uterus have no epithelial lining.

Of special interest are those cases of ovarian cysts in which in the course of typhoid fever infection of the cystic contents occurs with the corresponding organism.

For a consideration of the contents of hydatid cysts, pancreatic cysts, and hydronephrosis, see the respective sections in the second part of the book.

CHAPTER XI

BACTERIOLOGICAL APPENDIX

PREPARATION OF CULTURE MEDIA

Nutrient Bouillon.—Dissolve 6 to 8 grams of Liebig's beef extract together with 5 grams of sodium chloride and 10 grams of Witte peptone in about 100 c.c. of water by the aid of heat, stirring with a glass rod. Render the solution faintly alkaline to litmus (red paper should turn faintly blue, while the blue paper remains unchanged) by adding a fairly concentrated solution of sodium carbonate drop by drop. Or titrate 10 c.c. with $\frac{1}{10}$ normal alkali, using phenolphthalein as an indicator, to the point of the first pink which persists; estimate the corresponding amount of normal alkali which must accordingly be added to the remaining bulk of the fluid; add this and dilute to 1000 c.c.

Example.—10 c.c. required the addition of 10 c.c. of $\frac{n}{10}$ normal alkali. There remain 90 c.c. of bouillon; for each 10 c.c. in this, viz., 9, it is necessary to add 10 c.c. $\frac{n}{10}$ alkali; so in this case 90 c.c. Instead of using so much of the $\frac{n}{10}$ solution it is convenient to use 9 c.c. of the full-strength normal solution. This, however, is not necessary; the $\frac{n}{10}$ normal, in the amount mentioned, can be used, if *it* only is available.

If by any chance too much alkali has been added, use very dilute hydrochloric acid to return to the neutral point.

In any event test the final reaction with litmus paper and see to it that the reaction is slightly but distinctly alkaline while blue litmus paper remains unchanged Then filter into a liter flask, plug the mouth with cotton, and sterilize for one hour in the steam sterilizer. After that tubes are filled to the desired height ($1\frac{1}{2}$ to 2 inches) and again sterilized.

Glucose Bouillon.—This is nutrient bouillon to which 1 to 2 per cent. of glucose has been added.

Lactose Bouillon.—Nutrient bouillon, containing 1 to 2 per cent. of lactose.

Other carbohydrate bouillons contain corresponding amounts of material, inulin, mannite, etc.

Nutrient Gelatin.—6 to 8 grams of Liebig's beef extract, 5 grams of sodium chloride, and 10 grams of Witte peptone are dissolved in a liter of water, as in the preparation of nutrient bouillon. To this

solution 100 to 150 grams of gelatin are added, the latter broken up into small pieces. The mixture is boiled in an agate saucepan, stirring frequently so as not to burn the gelatin at the bottom. It is then neutralized as described above (preparation of nutrient bouillon), and clarified by the addition of the white of an egg beaten up in 50 c.c. of water. Before this is added the solution should be allowed to cool to 60° C. After this the boiling is continued for fifteen minutes, allowance being made for evaporation by the addition of a little water from time to time. The solution is then filtered. To this end no hot-water funnel or other artificial contrivance is necessary. The essential requisite is that the gelatin is in solution and has beeen actually boiling. The filter is wetted thoroughly before; if the first 4 c.c. should pass through turbid they are passed back. If the filtration should cease, the material in the funnel must be further boiled and the filtration continued thereafter.

The filtrate is received in a flask, plugged with cotton, and sterilized on three consecutive days in the Arnold sterilizer for fifteen to twenty minutes daily. Tubes, however, can be charged on the first day and the sterilization carried on in these.

Nutrient Agar.—This consists of nutrient bouillon, containing 1 to 1.5 per cent. of agar. The agar (10 to 15 grams) is cut into very small pieces and placed for twenty-four to forty-eight hours in 600 c.c. of water containing the 5 grams of salt required for the liter of bouillon. In the meantime the 6 to 8 grams of Liebig's beef extract and 10 grams of peptone are dissolved in 400 c.c. of water, neutralized as described (see Nutrient Bouillon), and sterilized. After soaking as indicated, the agar-salt mixture and the neutralized beef-peptone solution are poured together into an agate saucepan and the depth of the liquid measured; 300 c.c. of water are then added to allow for evaporation during the two hours and a half of active boiling which must follow. *During this process the liquid must not fall below its original bulk.* The white of an egg beaten up in 50 c.c. of water is then added (the liquid should be previously allowed to cool to 60° C., by setting the pan in a vessel with cold water), after which the boiling is continued actively for half an hour longer, when the agar is filtered through a previously prepared filter which has been well wetted. If the agar is well in solution the liter will pass through in little more than half an hour. If filtration should stop, the material must be boiled again and a new filter prepared. The agar can be filtered into tubes the same day or kept in a plugged flask; in either case it must be sterilized for three consecutive days in the steam sterilizer for fifteen to twenty minutes daily.

If agar slants are to be prepared, care must be taken not to fill the tubes too high. After their final sterilization they are laid down, slightly elevated at the open end, so that the agar forms a long slant; in this position they remain for some hours (over night).

Glycerin Agar.—This is nutrient agar containing 6 to 8 per cent. of glycerin. This is added after filtration and before sterilization.

Glucose Agar.—This is nutrient agar containing 1 to 2 per cent. of glucose. The glucose is conveniently dissolved in the beef extract-peptone portion.

Other carbohydrate agars contain corresponding amounts of material.

Litmus Agar.—This is ordinary agar which has been colored by the addition of a 5 per cent. solution of purified litmus; the agar should show a bluish color.

Litmus-carbohydrate Agar.—Litmus agar containing 1 per cent. of one of the various carbohydrates—dextrose, lactose, mannite, etc.

Hydrocele Agar (Cushing).—The fluid (hydrocele or ascitic) is obtained sterile, the locality of puncture being carefully sterilized by modern surgical methods, the sterile trocar covered at its external end with sterile gauze, so as not to be infected by the operator's hand, and the fluid collected in sterile flasks, the sterile stoppers being then replaced. When collected in this way it rarely becomes contaminated and may often be kept for months before using. This fluid is mixed with ordinary nutrient agar. A number of common agar slants are placed in the autoclave for five minutes. This liquefies the agar and at the same time thoroughly sterilizes the tubes and cotton stoppers. The slants are then put in a water bath at 55° C., so as not to coagulate the albumin when mixed with the agar. The stopper having been removed from a small flask of hydrocele fluid, the top of the flask is flamed and the albuminous fluid then poured into an agar tube (the top of which has also been flamed) in the proportion of a little more than 1 to 2. It is well to have as much of the hydrocele fluid as the future solidity of the medium will allow. Ordinary agar will allow not quite equal parts of the two. The stopper is then returned to the agar tube, which is immediately slanted.

Blood Agar.—Agar tubes are melted, as just described, and then placed in a water bath at 50° C. To each tube approximately one-half of a cubic centimeter of human blood is added. Agar and blood are well mixed and the tubes immediately slanted. Before use they should be incubated for twenty-four hours to see that they are sterile. The necessary blood may be obtained by aspirating a vein with a sterile syringe, containing a little 1 per cent. sodium citrate in saline, to prevent coagulation. Or one may use animal blood that has been sterilized according to the method of Bernstein and Epstein, as follows:

Sterilization of Blood.—400 c.c. of blood are collected in a sterile flask containing 30 c.c. of a 1 per cent. solution of ammonium oxalate (in distilled water) and 0.5 c.c. of formalin (standard 40 per cent. strength). The flask is agitated for a minute or two and then set aside

for one-half hour—the time which is required for sterilization. The blood is then distributed among a number of smaller flasks, and each specimen diluted with an equal volume of saline (0.9 per cent.). In this manner the formalin content is lowered to 1: 2400. After standing for twenty-four to forty-eight hours to give the formalin a chance to diffuse through the entire contents, the blood is sterile and ready for use. If not needed at once the flasks are sealed and stored on ice.

To prepare *blood agar* or *blood broth,* the writers recommend to mix 1 part of the diluted blood with 15 parts of the agar or broth, as the case may be. The final product thus has a formalin content of 1: 36000, which in nowise interferes with the growth of such organisms as the pneumococcus, the meningococcus, the gonococcus or the influenza bacillus.

Neutral Red Agar.—Agar, 2 per cent.; grape sugar, 0.3 per cent.; neutral red solution, 1 c.c. (saturated watery solution of Ehrlich's neutral red). Mix; sterilize.

Dunham's Solution.—This is common nutrient bouillon without the addition of Liebig's beef extract. Its reaction is neutral or slightly alkaline *per se,* and need hence not be corrected. The solution is filtered, tubes filled and sterilized, as in the case of bouillon.

Litmus Milk.—Fresh milk which has been freed from cream, as far as possible, is treated with tincture of litmus until it presents a distinct blue color. Tubes are filled with this and sterilized on two successive days for an hour at a time.

Litmus Whey.—To 500 c.c. of milk add 10 to 12 c.c. $\frac{n}{1}$ solution HCl to precipitate the casein. Neutralize with soda solution. Boil one to two hours. Let the precipitate fall to the bottom. Take 100 c.c. of fluid and add 5 c.c. litmus solution. Place in tubes; sterilize for from two to three hours at 100° C.

Potato Slant.—Large potatoes are selected. They are thoroughly scrubbed in running water and cylinders forced out with a large cork borer. They are cut square at the ends and then obliquely into two parts. The resultant wedges are kept over night in running water and the next day are placed in sterile tubes. The potato tubes are steamed for one hour.

Loeffler's Blood Serum.—3 parts of ox-blood serum are mixed with 1 part of nutrient bouillon containing 1 per cent. of glucose. Tubes are filled with this mixture and coagulated in a slanting position in the drying oven at a temperature a little above 90° C. It is important to raise the temperature to this point quite gradually. Here they remain until the slants are quite firm, after which they are sterilized in the steam sterilizer at 100° C. for fifteen minutes at a time, on three consecutive days.

The blood necessary for the preparation of the medium is procured at a slaughter-house. Care should be taken that it flows directly from the cut vessel into a suitable receptacle, which has

been previously sterilized. Museum jars are convenient for this purpose. After coagulation has set in the coagulum is carefully separated from the walls of the vessel with a sterile glass rod and the blood kept in a cool place (ice-box). The serum which separates out is pipetted off with a sterile pipette and placed in sterilized and plugged cylinders or test-tubes until required.

Two gallons of blood will approximately yield from 500 to 700 c.c. of serum.

Hiss' Serum-water Media.—The serum water is composed of beef serum 1 part and distilled water 2 or 3 parts. To this 1 per cent. of a 5 per cent. solution of highly purified litmus is added. The medium is heated for a few moments to 100° C., when 1 per cent. of either dextrose, lactose, maltose, saccharose, raffinose, dextrin, glycogen, inulin, mannite, or dulcite is added. Tubes are then filled and sterilized on three consecutive days by steam at 100° C. for fifteen minutes at a time.

The Drigalski-Conradi Medium. 1. *Agar Preparation.*—To 3 pounds of finely cut beef add 2 liters of water. Allow it to stand until next day. The expressed meat juice is boiled for one hour and filtered. Add 20 grams of Witte peptone, 20 grams nutrose, 10 grams NaCl; boil one hour, now add 70 grams bar agar, then boil three hours (or one hour in autoclave), render slightly alkaline (indicator litmus paper). Filter; boil half an hour.

2. *Litmus Solution.*—Litmus solution (Kubel and Tieman) 260 c.c., boil for ten minutes; add milk sugar (chemically pure) 30 grams. Boil fifteen minutes.

3. Add the hot litmus-milk-sugar solution to the liquid agar solution cooled to 60° C. Shake well. Render it again faintly alkaline. The color of the froth is a good indicator. Add then 2 c.c. of hot sterile solution of 10 per cent. water-free soda; further add 20 c.c. of a freshly prepared solution of 0.1 gram crystal-violet B. (Höchst) in 100 c.c. of warm water (distilled).

One has now a meat-water peptone-nutrose agar with 13 per cent. litmus and 0.01 pro mille crystal violet. This can be poured directly into plates and the remainder kept in 200 c.c. flasks.

The Malachite-green Medium of Lentz.—Preparation: 3 pounds of lean beef, finely divided, are macerated with 2 liters of water for sixteen hours. The extract is expressed, boiled for half an hour, filtered, then 3 per cent. agar added and boiled for three hours; then add 1 per cent. peptone, 0.5 per cent. NaCl, and 1 per cent. nutrose (this may be omitted). The mixture is brought to the litmus-neutral point by soda solution, boiled one hour, and filtered through linen. The reaction of the finished agar is sometimes distinctly acid. It is filtered into small flasks of 100 to 200 c.c.

Before the addition of the malachite green the hot agar is tested with neutral litmus paper and so far alkalinized with sterile soda

solution until the strip is distinctly red-violet. This reaction point corresponds in agar, without nutrose, to an alkalescent degree of 1.8 per cent. normal soda below the phenolphthalein-neutral point; if the agar contains nutrose, which remains neutral toward litmus, then the alkaline reaction corresponds to 3.5 per cent. normal soda solution below the phenolphthalein point.

To 100 c.c. of the hot agar 1 c.c. of a 1 to 220 solution of malachite green (crystal, Höchst) (the solution is stable for ten days) is added; the agar thus contains 1 in 22,000. With this concentration of malachite green (crystal) the growth of the usual kinds of *B. coli*, as well as many alkali-forming organisms, is greatly diminished and practically prevented.

The *B. typhosus* growth is also diminished, but only so far that after twenty-four hours the colonies can be recognized with the naked eye; they are then the size of a particle of sand, while, after a longer period in the incubator, in two to four days, larger, stronger colonies appear which color the agar yellow.

The finished agar is poured at once into Petri dishes in layers 2 mm. thick. The plates are allowed to remain open until all the steam has evaporated and the agar is stiff. It is essential that the surface of the plates should be quite dry and firm. Contamination by air organisms does not occur on account of the aniline dye present in the culture media.

Petroff's Medium.—This is used to isolate the tubercle bacillus from the various secretions and excretions (see Tubercle Bacillus). It is composed of two parts of egg (white and yolk), one part of meat juice, and gentian violet in sufficient quantity to give a proportion of 1 in 10,000. The dye is left out, if it is merely desired to cultivate the organism after its isolation.

To Prepare the Meat Juice.—500 gm. of beef or veal are placed in a saucepan with 500 c.c. of a 15 per cent. solution of glycerin in water. Twenty-four hours later the meat is squeezed in a sterile meat press and collected in a sterile beaker.

The eggs are prepared by sterilizing the shells by immersion for ten minutes in 70 per cent. alcohol, or by scalding them with hot water. The contents are broken into a sterile beaker, well stirred and passed through sterile gauze.

The gentian violet is used in the form of a 1 per cent. alcoholic solution and added in the required amount. Test tubes are charged with about 3 c.c. each of the final product, placed in a slanting position in a Koch apparatus and heated, the first day at 85° C. until the medium is solidified, and on the second and third day for not more than one hour at 75° C.

During incubation, after inoculation, the tubes are paraffined so as to guard against evaporation.

Besredka's Medium.—This medium is recommended by Besredka to secure an abundant growth of tubercle bacilli for the preparation of a suitable antigen for complement-fixation work in the diagnosis of tuberculosis. It is composed of a mixture of white of egg, yolk and bouillon. The individual components are prepared as follows:

1. Solution of white of egg. White of egg is stirred with 10 volumes of distilled water. The resultant liquid is opalescent and contains many small white flocculi in suspension. These are removed by filtering the material through a thin layer of cotton, when the filtrate is heated to 100° C., refiltered, tubed in portions of 20 c.c. each and sterilized for twenty minutes at 115° C.

2. The yolk solution is prepared by mixing yolk in 10 volumes of distilled water and clarifying with normal alkali (approximately 1 c.c. for 100 c.c. of the emulsion, an excess of the alkali being carefully avoided). After clarification the solution is still slightly opaque, especially when viewed in thick layers (Trial titration). It is then heated to 100° C., filtered, tubed in 20 c.c. lots and sterilized for twenty minutes at 115° C.

3. Preparation of the broth: 500 to 750 gm. of beef or veal are passed through a meat hasher, placed in 1000 c.c. of water and kept at a temperature a little lower than boiling for thirty minutes, when the mixture is filtered, rendered alkaline to litmus, heated to 120° C., filtered through a double layer of filter paper and bottled in portions of 100 c.c. in flasks of 150 to 200 c.c. capacity. These are finally sterilized at 115° C. for twenty-five minutes.

The three solutions are then mixed in the proportion of 20 c.c. of the albumen solution and from 5 to 20 c.c. of the yolk solution (nearer 5 is better than nearer 20) for 100 c.c. of the broth.

THE PATHOGENIC ORGANISMS

Staphylococcus Pyogenes.—The *Staphylococcus pyogenes aureus* (Fig. 192) occurs in the form of spherical bodies, averaging about 0.8μ in diameter, which readily stain with the basic anilin dyes, as also with Gram's method. They usually occur in clumps, but may also be seen in pairs and in short chains. The organism grows on all culture media, and in the presence of oxygen gives rise to the formation of an orange-yellow pigment. This is particularly well seen when the organism is grown on potato. Gelatin is rapidly liquefied. It coagulates milk with acid reaction and clouds bouillon. The *Staphylococcus pyogenes albus* and *citreus* differ from the aureus by the absence of pigment in the first and by the formation of a lemon-yellow pigment in the second.

Streptococcus Pyogenes.—The streptococcus may occur in pairs or in chains of pairs, composed of from four to twenty individual members. The size of the individual organism is somewhat smaller

than that of the staphylococcus, but may vary in one and the same chain. It can be readily stained with the usual basic anilin dyes in aqueous solution, and also with Gram's method. While it grows more or less readily on all of the common culture media, it has a distinct preference for those containing animal proteins (milk, serum agar, serum bouillon, blood agar). On agar and gelatin it forms tiny, grayish, almost translucent colonies. As a rule it does not liquefy gelatin. Plain broth cultures are usually faint, while in serum broth a more abundant growth takes place. Litmus milk is acidified and coagulated. The organism ferments the monosaccharids and disaccharids, but unlike the pneumococcus it is without effect upon inulin.

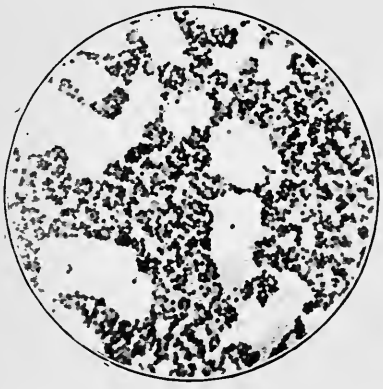


FIG. 192.—Staphylococcus pyogenes aureus. × 1000. (Herzog.)

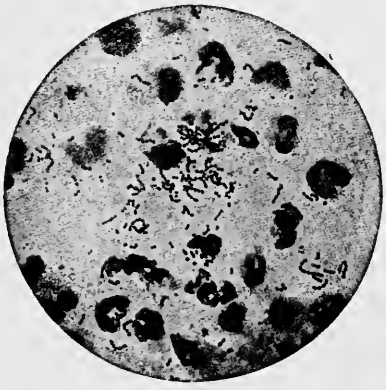


FIG. 193.—Streptococcus pyogenes. × 800. (Fränkel.)

Several varieties are recognized. The most notable from the stand-point of human pathology are the Streptococcus viridans, Streptococcus hemolyticus, and Streptococcus mucosus.

The Streptococcus viridans is characterized by the fact that its colonies on blood agar plates present a green color and are surrounded by a narrow pale-green halo (owing to the formation of methemoglobin).

The Streptococcus hemolyticus, on the other hand, while giving rise to a clear hemolytic zone around its colonies, does not produce a green color. The organism is dissolved by the addition of bile, while Streptococcus viridans is not affected in this manner, unless the bile is added in excess.

Streptococcus mucosus, in contradistinction to the two preceding groups, is provided with a capsule.

The Pneumococcus (Diplococcus lanceolatus).—The individual organism (Fig. 194) is capsulated, and usually occurs in pairs, arranged end to end or in short chains. At times, however, the chains

are quite long, and then it may be difficult to distinguish it from streptococci. It is easily stained with the common anilin dyes. In order to differentiate the capsule, the method suggested by Bürger should be employed. To this end smears are prepared as usual. As soon as the edges begin to dry they are covered with Müller's fluid, saturated with bichloride of mercury (ordinarily about 5 per cent.). The specimens are gently warmed over the flame for about three seconds (using cover-glass smears), rapidly washed in water, flushed once with alcohol (80 to 95 per cent.), and then treated with ordinary tincture of iodin for one or two minutes. The iodin in turn is thoroughly washed off with alcohol and the preparations dried in the air. They are then stained for two to five seconds with gentian anilin water (anilin oil, 10 c.c.

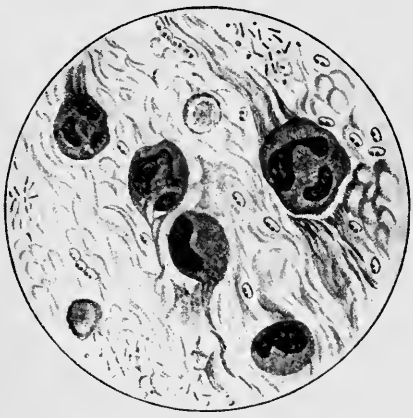


FIG. 194.—Pneumococcus, showing capsule.

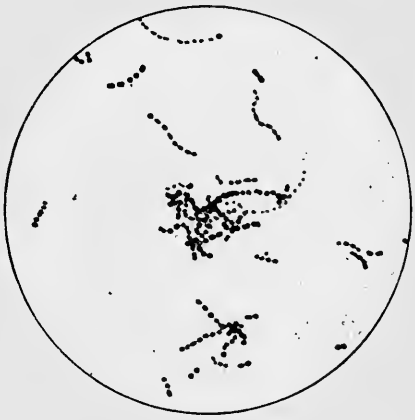


FIG. 195.—Pneumococcus from bouillon culture, resembling streptococcus. (Park.)

water, 100 c.c.; shake, filter, and add 5 c.c. of a saturated alcoholic solution of gentian violet; or 10 per cent. aqueous fuchsin solution, viz., saturated alcoholic solution of fuchsin, 10 c.c., and water, 100 c.c.). Washing with a 2 per cent. aqueous solution of salt completes the process. The preparations are examined in a drop of the salt solution and ringed with vaselin.

With this method there is visible a refractile, deeply staining, regularly outlined, narrow, elliptical capsule membrane, separated from the diplococcus by a clear area of capsular substance which either remains unstained or takes a faint color.

If smears are to be made from cultures or from material which in itself is essentially non-albuminous. Bürger directs that a drop of blood serum diluted with an equal amount of saline solution should be placed upon the slide or cover, and that the smear be made in this. Epstein finds that albumin water (egg albumin shaken with an

equal volume of water or normal salt solution) works just as well and will keep for two or three weeks.

Hiss' method also furnishes good results. The dry smears are fixed by heat and steamed for a second with a saturated alcoholic solution of gentian violet or fuchsin. The dye is washed off with a 20 per cent. copper sulphate solution, when the specimens are blotted dry (not washed) and mounted in balsam.

The organism grows best on human blood or serum culture media, but also does well in milk, which it usually coagulates by acid production. Very satisfactory results will be obtained, when it is desired to cultivate the organism from the blood of cases of pneumonia, if 5 c.c. of the blood are added to a flask containing from 75 to 125 c.c. of broth, prepared from fresh meat, to which from 20 to 25 c.c. of normal sodium hydrate solution have been added per liter. In this medium active growth is obtained within twenty-four hours. On ordinary agar, in gelatin and broth, its growth is feeble, and usually it dies out after a few transplantations. If not, it rapidly looses its virulence, which can then be restored only by passage through a suitable animal (mice, rabbits). The individual colonies on agar or serum agar resemble those of streptococci, but are usually more transparent.

To differentiate the organism from streptococci, Hiss' serum water containing inulin is most convenient. This is usually fermented by the pneumococcus with coagulation of the serum, while streptococci do so only rarely.

The Gonococcus.—The gonococcus (Plate XXVI) occurs in the form of small oval or coffee-bean-shaped granules, grouped in twos and fours resembling a German biscuit; the individual cocci measure about 1.25μ in length by 0.7μ in diameter. As a rule, they are found inclosed within pus corpuscles and epithelial cells; but they may also occur free in the pus obtained from the urethra, in the vaginal discharge, and more rarely in urinary sediments, as in cases of complicating prostatitis, peri-urethritis, etc. In cover-glass specimens account should be taken only of those organisms which are inclosed within cellular elements, as these alone may be regarded as characteristic. To this end a drop of the discharge is spread in a thin layer upon a slide or a cover-glass, dried in the air, and fixed by passing three or four times through the flame of a Bunsen burner. The specimens may then be stained with any one of the basic aniline dyes. In my laboratory the eosinate of methylene blue is almost exclusively used for this purpose. The organisms are thus colored blue, while the granules of eosinophilic leukocytes, which may be present at the same time, appear a bright red or a brownish red. After five minutes the excess of stain is washed off, the preparations are rinsed in water, dried with filter paper, and examined with a high power.

The gonococcus is decolorized by Gram's method, and can in this manner be distinguished from certain other organisms that may be present. Of the four kinds of diplococci which may be found in urethritis besides the gonococcus, only two forms are similarly decolorized besides the gonococcus, and these two are rarely seen. We may conclude that in 95 per cent. of all cases Gram's method permits a definite conclusion as to the presence or absence of the true organism. *Gram's method is* best employed in the modification suggested by Weinrich: The preparations are fixed by drawing through the flame of a Bunsen burner and are then stained for from one to two minutes in Fränkel's carbol-gentian-violet solution (10 parts of a saturated alcoholic solution of gentian violet to 90 parts of a 2.5 per cent. solution of carbolic acid). Without washing they are placed for one to three minutes in Lugol's solution (1 gram of iodin, 2 grams of potassium iodide, and 300 c.c. of distilled water), and again without washing in absolute alcohol, until the alcohol ceases to extract color (about one and one-half minutes); they are now washed in water, counter-stained with Bismarck brown, washed, dried, and mounted. The Bismarck-brown solution is prepared as follows: 3 grams of the dye are dissolved in 70 c.c. of hot water; 30 c.c. of 96 per cent. alcohol are added; the mixture is well stirred and filtered.

The organism grows best on blood and hydrocele agar. The surface colonies are pale, grayish, translucent, and finely granular, with finely notched borders. In bouillon and blood serum mixed it forms a membrane, while the fluid remains clear. Some cultures (on hydrocele agar) will maintain a vigorous growth after numerous transplantations, while others grow only two or three times, or indeed, once only.

The Meningococcus Intracellularis.—The organism is a diplococcus (Fig. 196) of about the same size as the gonococcus. It is readily stained with the usual dyes, and decolorized by Gram's method. Short chains of from four to six may at times be seen, as also tetrads and peculiarly swollen forms which are much larger than the usual forms. Cultivation is difficult and the organism quickly dies out. It grows best upon blood agar and Löffler's blood-serum mixture, forming round, whitish, shining, viscid-looking colonies, with smooth, sharply defined outlines, which may attain a diameter of from 1 to 1.5 mm. in twenty-four hours. Their cultivation upon plain agar, glycerin agar, and in bouillon is less reliable. I have obtained excellent results by placing a few cubic centimeters of the cerebrospinal fluid in blood-serum tubes and found that the organisms multiplied far more actively in the fluid over the medium than in any other way.

In order to obtain the best results, it is necessary to use large amounts of the exudate, and to make a number of cultures, as many

of the organisms are usually dead, or at least will not grow. In ordinary cover-slip preparations they are often numerous, and are mostly found inclosed in the polynuclear leukocytes. Their number then varies considerably. On the one hand, only one or two may be present in a cell, while in others they may be so closely packed as to obscure the nucleus. On one occasion I examined a specimen in which the organism was present in groups composed of hundreds, but this is rare.

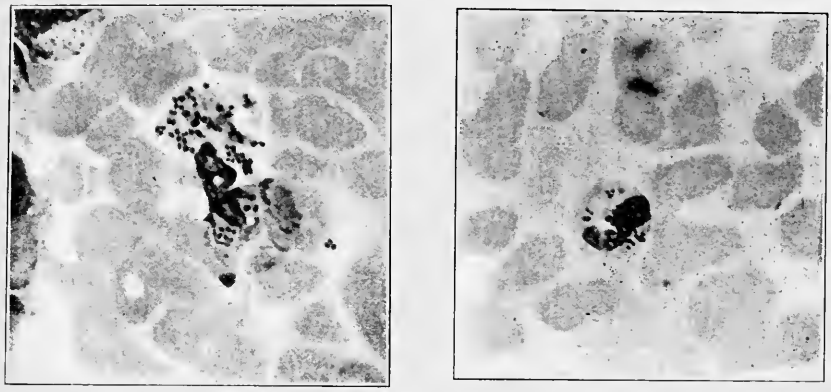


FIG. 196.—Diplococcus meningitidis intracellularis.

The Diphtheria Bacillus.—The bacillus (Figs. 197, 198 and 199) is non-motile and varies in size and shape, its average length being from 2.5 to 3μ, its breadth from 0.5 to 0.8μ. Its morphological characteristics are so peculiar as to render its identification upon cover-slip preparations and in sections of the diphtheritic membrane an easy matter in most cases.

Sometimes the organism appears as a straight or slightly curved rod; but especially characteristic are irregular and often bizarre forms, such as rods with one or both ends terminating in little bulbs, and rods apparently broken at intervals, in which short, well-defined, round, oval, or straight segments can be made out. Very commonly two organisms lie together, forming an obtuse angle, or numbers of them may be observed lying side by side.

The organism grows best on Löffler's blood serum; upon this it develops so much more rapidly than other organisms which are usually present in the secretions of the mouth and throat, that after six to eight hours' incubation at 34° to 35° C. it often forms the only colonies that attract attention. Smears are then made and stained according to Neisser's or Löffler's method.

In the absence of blood serum, bouillon, nutrient gelatin, nutrient agar, glycerin agar, and potato may be employed. Coagulated egg

albumin, as pointed out by Booker, and milk are also good media. But it is to be noted that the "typical" staining effect with Neisser's method is commonly only obtained if the organism has been grown on ox-blood serum, and if the growth is not older than twenty-four hours. The colonies are large, round, elevated, and grayish white in color, with a centre that is more opaque than the slightly irregular periphery. The surface of the colony is at first moist, but after a day or two it assumes a dry appearance.

FIG. 197.—Characteristic forms of diphtheria bacilli from blood-serum cultures, showing clubbed ends and irregular stain × 1100 diameters. (Park.)

FIG. 198.—B. diphtheriæ. Forty-eight hours' agar culture. Thick, medium-clubbed rods and moderate number of segments. One year on artificial culture media. × 1410 diameters. (Park.)

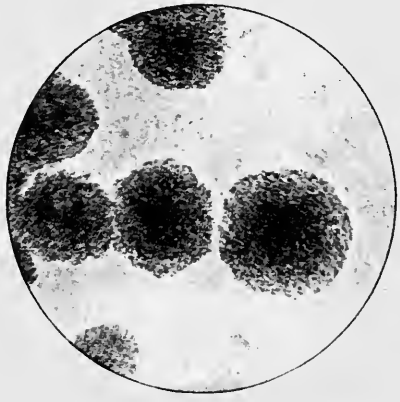


FIG 199.—Colonies of diphtheria bacilli. × 200 diameters. (Park.)

According to Knapp the true bacilli, in contradistinction to the pseudodiphtheria bacilli, will ferment dextrose and maltose. The Bacillus xerosis will do the same. In contradistinction to the diphtheria organism the Bacillus xerosis will ferment cane sugar; the former, in contradistinction to the xerosis will ferment dextrin. The fermentation tests must be made with the litmus serum-water

media of His. Results after twenty-four hours' growth at 37° C.: Pseudodiphtheria—none of the sugars fermented; media remain blue. Diphtheria—dextrose, mannite, maltose, and dextrin fermented; media red and coagulated. Saccharose not fermented. Xerosis bacillus—dextrose, mannite, maltose, and saccharose fermented with acid production; media red and coagulated. Dextrin not fermented. The Bacillus xerosis, moreover, forms a very thin scum or pellicle on the surface of the media which is absent with the other bacteria.

To demonstrate the organism in the diphtheritic exudate a piece of membrane is scraped from the tonsils, the soft palate, or pharynx by means of a pair of forceps, a stout platinum loop, or a cotton swab. Cultures are prepared from this and smears are made on slides or cover-glasses in the usual manner. After fixation by passing them several times through the flame of a Bunsen burner they are stained for five to ten minutes in Löffler's alkaline solution of methylene blue, which consists of 30 c.c. of a concentrated alcoholic solution of methylene blue in 100 c.c. of an aqueous solution of potassium hydrate (1 to 10,000). They are then rinsed in water, dried, and examined with a $\frac{1}{12}$ oil-immersion lens.

A rapid method of staining, and one which also gives satisfactory results, is suggested by Neisser. The organism is grown on ox-blood serum and examined after nine to twenty-four hours. The air-dried smears are placed for one to three seconds in a solution composed of 20 c.c. of an alcoholic solution of methylene blue (1 gram to 20 c.c. of 90 per cent. alcohol), 950 c.c. of distilled water, and 30 c.c. of glacial acetic acid. They are then washed in water, stained for three to five seconds in a 0.2 per cent. hot and filtered aqueous solution of vesuvin, again washed off, dried in the air, and mounted in balsam. The bacilli are brown and have in their interior two to four blue granules which are usually located near the poles.

The following method also may be employed, as suggested by Schauffler. The staining reagent has the following composition:

Filtered solution of Löffler's methylene blue	10.0 c.c.
Filtered solution of pyronin (0.5 gram to 10 c.c. of water) .	1.5 c.c.
Acid alcohol (3 c.c. of 25 per cent. hydrochloric acid to 97 c.c. of absolute alcohol)	0.5 c.c.

Cover-glass specimens are stained for one minute; they are then washed in running water and mounted in balsam as usual. The bacilli are stained blue, the polar bodies a bright ruby red.

Pseudodiphtheritic bacilli are said to take only the blue stain with this method.

The Tubercle Bacillus.—To demonstrate the tubercle bacillus in the sputum Gabbett's method or that of Weigert-Ehrlich or Ziehl-Neelsen is best employed.

Gabbett's Method.—Bits of purulent or hemorrhagic material, or if present the cheesy particles previously referred to, are spread on slides in thin layers. These are dried in the air and fixed by being passed a few times through the flame of a Bunsen burner or an alcohol lamp. The specimens are covered with a few drops of carbol-fuchsin solution and heated to boiling for one-quarter to one-half minute. The solution is composed of 1 part of fuchsin dissolved in 100 parts of a 5 per cent. solution of carbolic acid and 10 parts of absolute alcohol. The excess of the staining fluid is drained off and replaced, without washing with a solution composed of 2 parts of methylene blue in 100 parts of a 25 per cent. solution of sulphuric acid. After a minute or two they are washed in water, dried, and examined directly in oil.

It has been suggested by Pagani to use lactic acid instead of sulphuric acid, in order to avoid a too energetic decolorization. He claims that excellent results are obtained if the second solution of Gabbett is replaced by the following: Water, 50 c.c.; alcohol, 50 c.c.; lactic acid, 2.5 grams; and methyl blue to saturation. The cover-glass specimens or slides are immersed in this solution for from fifteen to twenty seconds while gently agitating.

Gabbett's method of staining is very convenient, and is the one most generally employed. The smegma bacillus, however, is also stained.

The Weigert-Ehrlich Method.—Dried specimens are prepared, and stained for twenty-four hours with a solution of fuchsin in anilin water. The staining fluid is prepared as follows:

A test-tubeful of water is shaken with about 20 drops of pure anilin oil and, after standing for a few minutes, filtered through a moistened filter. To this solution a few drops of a concentrated alcoholic solution of fuchsin or of methyl violet are added until the mixture becomes slightly cloudy—*i. e.*, until a metallic luster is noted on the surface. After twenty-four hours the preparations are washed with water in order to remove an excess of staining fluid. They are then immersed for several seconds in a dilute solution of nitric or hydrochloric acid (1 to 6, 1 to 3, or 1 to 2), and washed again with water or with absolute alcohol. At this time the specimens should have a faintly red or violet color. They are then dried, and mounted as usual.

If it is desired to use a counterstain, Bismarck brown, or methylene blue in watery solutions may be used. Into such a solution the specimen is placed after treatment with nitric acid and washing in water. It remains for about two minutes, and is then washed, dried, and mounted as above.

Ziehl-Neelsen's Method.—A mixture of 90 parts of a 5 per cent. solution of carbolic acid and 10 parts of a concentrated alcoholic solution of fuchsin is used. The procedure is the same as that

described under the Weigert-Ehrlich method. It is usually not necessary to stain the preparations for twenty-four hours, however; as a rule it is sufficient to place a few drops of the staining fluid upon the preparation and to heat over the free flame as described when the specimen is decolorized as before. In this manner excellent results may be obtained in a few minutes.

Stained according to one of these methods, the bacilli appear as rods, measuring about 1.5 to 3.5μ in length by 0.2μ in breadth (Plate XX, Fig. 1). Much larger specimens may, however, also be seen, up to 11μ in length. The shortest forms are commonly straight; the common types are usually slightly curved. They may occur joined in chains of two or three, and branching forms have also been observed. Occasionally one may see a couple of organisms, each bent to a crescent, linked in the form of the letter S. Very commonly they are beaded, and it is possible to make out from 1 to 8 clear spaces in an organism, separated by round or rod-shaped granules, which are deeply stained and appear to lie in a lightly staining capsule. The small hyaline bodies were once regarded as spores, but it is more likely that they are vacuoles. Sometimes bacilli are seen which have club- or knob-shaped enlargements at the extremities. These enlargements likewise have been viewed as spores, while others look upon them as products of degeneration. When present in large numbers the bacilli are often seen in clumps, as though they had been agglutinated, but in every specimen isolated organisms are also found scattered through the field; or, two, or three in groups.

Cultivation of the Tubercle Bacillus.—While the tubercle bacillus may be cultivated from sputum, feces, urine, pus, etc., by treating the material in question with antiformin, washing free from the latter and then planting the final centrifugate upon potato or blood serum, better results are obtained with *Petroff's method.* In the case of sputum, equal parts (about 5 c.c.) of fresh material are mixed with 3 per cent. sodium hydrate, well shaken and incubated until the material has become hemogeneous. It is then neutralized with normal hydrochloric acid (to sterile litmus), centrifugalized, and the sediment inoculated into several test tubes containing Petroff's medium (which see). The gentian violet inhibits the growth of contaminating organisms and is essential. Macroscopic growth occurs by the seventh to the fourteenth day. Subcultures may then be prepared either on Petroff's medium without the gentian violet or on glycerin agar (6 per cent.). The temperature of the incubator should fluctuate as little as possible—37° to 38° C. Below 30° and at a temperature higher than 42° C. the organism does not grow. The individual colonies appear like small, dry scales, which gradually coalesce and form a wrinkled film of a dull, whitish color. Older cultures present a brownish or grayish-brown color. Sunlight rapidly kills the tubercle bacillus.

To isolate the tubercle bacillus from the feces the latter are diluted with three volumes of water, mixed well and filtered through several thicknesses of gauze. The filtrate is saturated with sodium chloride and allowed to stand for half an hour. The idea of salting is to so change the specific gravity that the bacteria will all float on top. The resulting film is removed, mixed with an equal volume of 3 per cent. sodium hydrate, shaken well and incubated for three hours at 37° C., shaking from time to time. The process is then continued as in the case of sputum (see above). Growth occurs more slowly than with the latter, viz., in from two to three weeks. While the results on the whole are satisfactory, contamination is more apt to occur than with the sputum.

The Bacillus of Dysentery.—This organism is now generally regarded as the specific cause of the common form of acute dysentery which prevails not only in the tropics, but also in the United States and Europe. It was discovered by Shiga in Japan in 1897, and is identical with the organism obtained by Flexner and Strong in the Philippines and Porto Rico, by Vedder and Duval in the United States, and by Kruse in Germany. From the researches of Bassett and Duval it further appears that the same bacillus is also responsible for the common form of infantile summer diarrhea which prevails in warm countries.

In the United States the Flexner-Harris type is by far the most common in infantile cases. In the collection of 237 cases reported by Holt this type was found in 207, while the true Shiga bacillus was present in only 23; both organisms were found in 7 cases.

The bacillus in question is a short rod with rounded ends, and resembles the typhoid bacillus and most members of the colon group. It is probably non-motile so far as active locomotion is concerned, but it is possessed of a high degree of molecular motion. It stains with the usual basic dyes and is decolorized by Gram's method.

Upon gelatin plates at room temperature there appear, after a few days, small round dots, which, magnified under low powers, are slightly yellow and finely granular. After a few days they increase in size; the middle portion of the colonies then appears darker under a low power, while the outer zone appears brighter and more seed-like. The superficial and deeper colonies show no marked variation. In stab cultures on gelatin a whitish strand forms the whole length of the stab. The gelatin is not liquefied.

After twenty-four hours in the incubator single colonies upon slanted agar appear moist, bluish, and partially translucent. After two days they present a combination of a middle dark and a peripheral bright, sharply defined zone.

The growth on glycerin agar is slightly more abundant than on ordinary agar. The organism grows on blood serum without liquefying it.

In the stab cultures on glucose agar there is formed along the whole line of the puncture a thick, gray-white strand without the development of gas. Upon potato after twenty-four hours in the incubator there is hardly any perceptible growth; only the surface appears slightly shiny. After two days this changes to a yellow brown. In the course of a week the growth is heavier and of a deeper brown color. Bouillon cultures show after a day in the incubator a somewhat intense cloudiness, with a moderate precipitate. No pellicle is formed on the surface. No indol reaction is present. Litmus milk after twenty-four hours appears reddish; otherwise, however, it undergoes no change. The milk never coagulates.

The bacillus is pathogenic for mice, rabbits, and guinea-pigs. It is agglutinated by the patient's blood serum, and it is interesting to note that this reaction is obtained only with cases definitely known to have been infected with the microörganism in question.

Isolation of Shiga's Bacillus from the Feces.—The fecal matter is collected on a sterile pad, or, still better, obtained from the rectum by curettage. A bouillon culture is prepared, and from this agar tubes are inoculated *as soon as possible.* The agar should be just acid to phenolphthalein (slightly alkaline to litmus), and is plated at once. Ten plates, variously diluted, are conveniently used. After twenty-four hours in the incubator at 37° to 38° C. all colonies are marked on the plates which have developed by that time. The plates are returned to the incubator. After further twenty-four hours tubes of glucose agar and litmus-mannite agar are inoculated from those colonies which have grown in the second twenty-four hours—*i. e.*, those colonies which have not been marked. At the end of another twenty-four hours in the incubator all those tubes are rejected in which fermentation has taken place. From those tubes in which this has not occurred, litmus milk, litmus mannite, and bouillon are inoculated. The Shiga bacillus will at first render the milk slightly acid, but later it becomes alkaline. Litmus mannite remains unchanged with the Shiga strain, while the Flexner-Harris type (the American acid type) turns it red. Ultimate identification is made by the agglutination test in various dilutions (1 to 50 to 1 to 100), reading the results after two hours.

The Typhoid Bacillus.—In pure culture typhoid bacilli present the following features: They occur in the form of rods of almost one-third the size of a red blood corpuscle, or in threads composed of several rods joined end to end (Figs. 200 and 201). Their ends are rounded; their length is equivalent to about three times their breadth. They are actively motile and provided with polar as well as lateral flagella. They grow very readily on bouillon-peptone gelatin, and after twenty-four hours colonies begin to appear. When slightly magnified, these present a faintly yellowish color; macroscopically they are barely visible. The organism does not form spores, but when

kept at a temperature of 37° C., and especially when grown on media colored with phloxin red or benzopurpurin, polar bodies are observed which were formerly mistaken for spores. Gelatin is not liquefied; the growth is white and delicate, both along the line of the stab and on the surface. Cultivation in glucose bouillon, or glucose agar, does not give rise to the formation of gas, but after twenty-four hours the entire fluid becomes turbid. Milk is rendered very feebly acid, but is not coagulated. No indol reaction is obtained when the organism is grown on peptone-containing media. On potato a very faint, whitish, almost invisible growth takes place. When grown on gelatin or agar that has been colored with neutral red, the typhoid bacillus causes no change in color. Absolute identification is possible by means of Pfeiffer's agglutination test. (See Widal's reaction.)

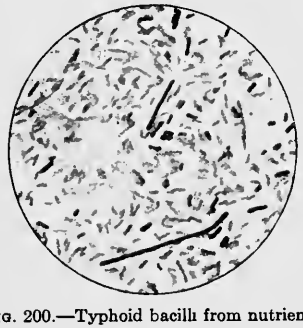


FIG. 200.—Typhoid bacilli from nutrient agar. × 1100 diameters. (Park.)

FIG. 201.—Typhoid bacilli from nutrient gelatin. × 1100 diameters. (Park.)

Isolation from the Feces.—In the feces the typhoid bacillus can only be demonstrated by cultural method which will enable its separation from other members of the colon group. To this end many different methods have been suggested.

Combined Malachite-green Method of Lentz and the Method of v. Drigalski and Conradi.—This method is probably the most useful and extensively employed abroad. The media are prepared as described elsewhere. (See Media.) Two plates of Drigalski's medium are prepared in large Petri dishes, using 20 to 25 c.c. of the medium for each plate (15 to 20 cm. diameter); these are left uncovered until the steam has evaporated and the agar is quite firm. Contamination by the organisms of the air does not occur owing to the presence of the cresyl violet in the medium. The malachite-green medium is already plated when made. (See Media.) The stool is stirred up well with a small amount of sterile normal salt solution. Of this material, about 0.5 c.c. is placed on the green plate and smeared over its surface with a glass rod, which is conveniently bent at an angle about one inch from the end. Without

sterilizing, the same rod is then smeared over the first Drigalski plate and hence over the second. After this all three are allowed to become perfectly dry by standing open in the air, when they are incubated for twenty to twenty-four hours. Plates 2 and 3 are now examined with a hand lens, placing them, if possible, in such a position that light reflected from a wall falls upon them. The colon colonies are more or less red in color, not transparent, and measure 1 to 3 mm. in diameter. The typhoid colonies are bluish with a violet shade and resemble drops of dew. If such are found they are further identified as follows: A tiny bit of the colony is placed on a slide and mixed with a drop of a highly active hundredfold dilution of typhoid (viz., paratyphoid serum. Agglutination may be observed with a hand lens or a low power of the microscope. If this occurs further tests are made by inoculating ordinary agar, litmus whey, and neutral red agar. (See Culture Media.)

If no colonies are found on the Drigalski medium which resemble typhoid bacilli, the green plate is flooded with sterile normal salt solution, gently agitated, and set aside for a few minutes. In this manner the typhoid and paratyphoid colonies, which are more delicate than the colon colonies, come to be disseminated in the fluid, while the latter sink to the bottom. With the glass spatula two more Drigalski plates are then prepared from the salt solution, incubated for twenty to twenty-four hours, and examined as described.

If urine is to be examined in the place of feces, several drops are placed on the green plate and one drop only on the Drigalski plate. The procedure otherwise is the same.

Demonstration of the Typhoid Bacillus in the Blood.—8 to 10 c.c. of blood are withdrawn from one of the superficial veins of the arm, as described. Several Erlenmeyer's flasks, each containing 150 c.c. of bouillon, should be ready at hand. Blood is added to these in varying proportions: two receive 1 c.c. each, and two others, 2 c.c. each. In this way 1 to 150 and 1 to 175 dilutions are obtained. The flasks are well shaken and placed in the incubator for twenty-four hours. A hanging drop is then examined.[1] If negative the incubation is continued for another twenty-four hours. When the bouillon has become cloudy, subcultures are made in milk and glucose bouillon (see description of typhoid bacillus) and the organism further tested with an actively agglutinating serum (see below).

It is interesting to note, however, that the tendency to agglutination of freshly isolated typhoid bacilli is almost invariably much inferior to that of bacilli which have been maintained for a long time on artificial media. Courmont thus notes that they were commonly agglutinated with a dilution of 1 to 50 by a serum which agglutinated laboratory bacilli at 1 to 200.

[1] At first the bacilli are but little active, but on further cultivation and reinoculation their motility increases.

The Intermediates.—The intermediate group comprises the Bacillus enteritidis and similar organisms which have been isolated from cases of meat poisoning, certain varieties of gas-producing typhoid bacilli, the Bacillus psittacosis, Bacillus choleræ suis, Bacillus icteroides, and the so-called paracolon and paratyphoid bacilli. Unlike the typhoid bacillus, these organisms produce gas in glucose media, and unlike the colon bacillus, they do not ferment lactose, nor coagulate milk or form indol. Sera from typhoid fever either produce no agglutination or partial clumping only, and this only in low dilution.

Bacillus Coli Communis.—The Bacillus coli communis, while constantly present in normal feces, is described at this place, as modern investigations have shown that it may at times develop pathogenic properties. It has been found in the pus in cases of purulent perforating peritonitis, angiocholitis, pyelonephritis, etc.; it is frequently found infecting the bladder and the pelvis of the kidney, and, as indicated elsewhere, at times forming the nucleus of gallstones. It occurs in the form of delicate or coarse rods, measuring about 0.4 μ in length, which manifest a certain degree of motility, due to the presence of one or two polar flagella. The organism is stained by the usual anilin dyes, and is decolorized by Gram's method. The colonies upon gelatin closely resemble those of the bacillus of typhoid fever, forming small whitish specks *in* the gelatin, and delicate films with serrated borders *upon* the same medium, which, moreover, is not liquefied. On potato the organism forms a brownish pellicle, while the growth of the typhoid bacillus is nearly transparent. As in the case of the cholera bacillus, the nitroso-indol reaction can be obtained when the organism is grown upon peptone-containing media.[1] In solutions of glucose active fermentation takes place. Litmus milk is rendered acid and is coagulated. Important also is the behavior of the organism when grown on gelatin or agar that has been colored with neutral red; in contradistinction to the typhoid bacillus, the colon bacillus then causes an intense green fluorescence.

Bacillus Lactis Aërogenes.—The Bacillus lactis aërogenes (Escherich) closely resembles the organism just described, and may also at times develop pathogenic properties. It is seen quite constantly in the stools of sucklings, but may also be met with in those of adults. It occurs in the form of rather stout rods, which frequently lie in pairs, resembling diplococci. The organism is non-motile. Like the Bacillus coli communis, it is decolorized by Gram's method In plate cultures it forms a dense white film; in stab cultures a chain of white colonies resembling beads is seen. In the latter, moreover, if the stab is closed, bubbles of gas will be seen to form, which rapidly

[1] The test for indol is very conveniently made by adding a few drops of Ehrlich's dimethyl-amino-benzaldehyde solution (see Urine) to a culture of the organism in Dunham's solution which has grown for four or five days. On shaking, and especially on heating, a cherry-red color develops.

increase in number and size. Milk is coagulated in large lumps in twenty-four hours; at the same time the formation of gas is much more intense than in the case of the Bacillus coli communis.

Bacillus (Proteus) Vulgaris.—This organism, while usually regarded as non-pathogenic, should be numbered among the bacteria which may at times develop pathogenic properties. Baginsky and Booker have frequently found it in the stools in cases of infantile summer diarrhea. Escherich observed it at times in the meconium. Brudzinski examined the dyspeptic and fetid stools of a number of artificially fed infants in Escherich's clinic, and in all the cases found the proteus. Others have encountered it in inflammatory conditions of exposed surfaces, in appendicitis, in perforative peritonitis, and even in closed abscesses, either alone or in association with other bacteria (Welch). A mixed infection of the proteus with Löffler's bacillus has also been observed. The organism forms rods, measuring about 0.25 μ in diameter, while their length is variable; at times a more roundish form is observed; at others rods measuring from 1.25 μ to 3.75 μ in length, or even long threads. They are readily stained, but are easily decolorized by alcohol or Gram's method. Most characteristic is their growth upon nutrient gelatin. At the temperature of the room little depressions will be observed after six to eight hours, which are surrounded by a narrow zone of bacilli from which a thin, wide film, provided with irregular projections, extends over the culture medium. From this film islets become separated, which slowly extend over the gelatin and cause its liquefaction. The organism is motile. It decomposes urea and causes albuminous putrefaction. The nitroso-indol reaction is readily obtained in bouillon cultures. In boiled milk the organism grows well, while in fresh milk it develops only irregularly. In litmus milk the organism produces alkali and digests casein. It ferments dextrose and lactose with the production of acid and gas.

Bacillus Pyocyaneus.—The Bacillus pyocyaneus has repeatedly been isolated from the stools of dysenteric patients, and has been proved the cause of several epidemics. The organism in question is a small motile bacillus measuring from 1 μ to 2 μ in length by 0.3 μ to 0.5 μ in breadth. It sometimes occurs in short chains, but is usually single. It is stained with the common anilin dyes, and is decolorized with Gram's method. It grows on the usual culture media, and liquefies gelatin. In 2 per cent. glucose bouillon no fermentation takes place. Litmus milk is curdled in about forty-eight hours. Some varieties produce indol. Most characteristic is the production of certain pigments, viz., pyocyanin and a fluorescent, bluish-green pigment which is common to almost all varieties.

The Comma Bacillus.—The first detailed studies of the organisms found in cholera stools were made in 1883 by the members of the French and German commissions sent to Egypt to investigate the

nature of the dreaded disease. The result of their work was first published by Koch in his report to the Berlin Sanitary Office in 1883, and in 1884 by Strauss, Roux, Nocard, and Thuillier.

The clinical recognition of cholera Asiatica has now become a simple matter since Pfeiffer has demonstrated that the blood serum of cholera patients possesses the property of causing arrest of motility and agglutination of the specific bacilli. Ordinary bouillon cultures, however, can usually not be employed, as particles of the film when broken up may easily be mistaken for agglutinated bacilli. It is best in every case to make use of agar cultures sixteen to twenty-four hours old, and to prepare emulsions in bouillon or normal salt solution as occasion requires. The emulsion, moreover, should always be examined microscopically before use, so as to insure the absence of any conglomeration of bacilli. The blood is then diluted in the proportion of 1 to 10 or 1 to 15. If the test-tube method is employed, the tubes should be kept in the incubator (37° C.) for only one or two hours. Upon the slide the reaction is obtained in from five to twenty minutes. If no distinct agglutination is observed at the end of one hour, the diagnosis of cholera is rendered improbable. Dried blood retains its agglutinating properties for a considerable length of time, and may also be used for examination.

The comma bacillus (Fig. 202) is a slightly arched or half-moon-shaped little rod, and is somewhat shorter than the tubercle bacillus. Occasionally two are placed end to end with their convexities in opposite directions, thus presenting the appearance of the letter S. They are provided with flagella. Koch detected these bacilli in the intestinal contents and feces, but rarely in the vomited matter, in Asiatic cholera only. In the stools they at times occur in such numbers as to constitute pure cultures. In plate cultures kept at a temperature of 22° C. white colonies with serrated borders may be observed after twenty-four hours. The color of such a colony is slightly yellow or rose red, its central portion gradually assuming a deeper tint, and finally becoming liquefied. Upon agar plates the bacilli form a grayish-yellow, irregular, slimy coating, but do not liquefy the culture medium. In stab cultures, after twenty-four hours, a whitish color may be observed along the line of the stab; around this there is found a funnel-shaped depression, which gradually increases in size and apparently contains a bubble of gas. The upper

FIG. 202.—Cholera spirilla preparation from gelatin-plate culture of cholera. × 800 diameters. (Park.)

portion of the culture medium at the same time becomes liquefied while the lower portion remains solid for days. In a suspended drop spirochete-like spirals are observed at the margins, which often present as many as twenty distinct arches.

Closely related to Koch's comma bacillus is the *bacillus of Finkler and Prior*, discovered in 1884 and 1885. It is distinguished from the former by the following characteristics: It is larger and thicker than the comma bacillus; the colonies on gelatin plate cultures show equally round and sharp-edged forms, which present a granular appearance under a low or medium power, and are usually of a brown color. The organism liquefies gelatin very rapidly, a penetrating, excessively fetid odor being developed at the same time. In stab cultures the bacillus of cholera Asiatica forms a funnel-shaped depression, while the bacillus of Finkler and Prior forms a stocking-like depression.

The Bacillus of Influenza.—In cases of true influenza the corresponding bacillus is found in the bronchial sputum in large numbers. It is essentially characterized by its minute size, measuring only 0.2 μ to 0.3 μ in breadth by 0.5 μ in length (Fig. 203). The organisms occur for the most part singly, but may also form chains of threes and fours. In suitably stained specimens they may at first sight appear as diplococci, owing to the fact that the poles are stained more deeply than the intervening portion. Carbol fuchsin, diluted in the proportion of 1 to 10 with water, stains the bacillus very well and brings out the polar staining.

The organism is non-motile and forms no spores. It can be grown on media containing blood or serum (blood agar, hydrocele agar, Löffler's serum). Human blood and pigeon blood are the best. Growth, however, in any event is slight and occurs slowly. In order to cultivate the influenza bacillus from the sputum, this is collected in sterile cups and examined without delay. The sputa are washed in sterile bouillon or sterile normal salt solution and cultures made on blood agar. (Boggs recommends pigeon-blood agar or agar to which sterile fetal blood has been added.) Tiny, water-clear colonies then develop, as described by Pfeiffer. On the fetal-blood agar Boggs noted that involution forms appear earlier and in much greater number than when pigeon, rabbit, or adult human blood was used. Some of these forms are so large and irregular as to give at first sight the impression of a mixed infection.

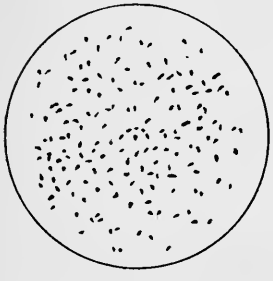


Fig. 203.—Influenza bacilli.

From the blood the organism is rarely obtained.

Influenza-like bacilli have been found in whooping cough sputa

by Spengler, Jochmann, and Krause, and more recently by Wollstein. The organism in question has been named the *Bacillus pertussis*, Eppendorf. According to Spengler the bacillus of Czaplewski and Hensel is only a contaminating pseudodiphtheria bacillus.

The Bacillus Pertussis.—To cultivate the whooping cough bacillus, the sputum masses coughed up after a paroxysm are washed in six successive beakers of peptone water and spread upon blood-agar plates prepared by mixing placental blood with melted agar. The predominating colonies are then small, transparent, dew-drop like, and not surrounded by a hemolytic zone, as in the case of the pneumococcus and streptococcus. Microscopically they appear as slightly raised, almost structureless droplets. After forty-eight hours the colonies show a slightly granular centre. The bacilli also grow in bouillon to which a drop of fresh or hemolyzed blood is added. On ascitic fluid agar, glycerin agar, Löffler's serum, plain bouillon, serum, broth, milk, and gelatin no growth takes place.

The organisms are not motile. They are short, plump, ovoid, with rounded ends, lying singly or in small groups, between the pus and epithelial cells of the sputum. They are decolorized by Gram's method. Somewhat larger forms are found in older cultures, and Spengler speaks of very long chains.

Wollstein obtained agglutination with the serum of the corresponding child in dilutions of 1 to 200 and occasionally of 1 to 500.

The Micrococcus Melitensis.—The organism in question is a coccus, measuring 0.3 μ in diameter; it occurs singly, in pairs, and sometimes in fours. Longer chains are not seen. It is motile. It is stained by the usual dyes and grows on nutrient agar and in broth. The colonies are usually not visible until the third day. At first their color is that of a transparent amber, while later they are opaque. Liquefaction does not occur.

The Plague Bacillus.—The organism in question (Fig. 204) is a short, thick coccobacillus, with rounded ends, measuring 1.5 μ to 1.75 μ in length by 0.5 μ to 0.7 μ in breadth. Examined in the hanging drop it is devoid of automobility. The polar regions are readily stained, while the interpolar area remains colorless. In many organisms a capsule can be made out by appropriate methods, but it is apparently not a constant feature. Oftentimes the form of the organism deviates from the normal. It may thus resemble a coccus on the one hand, while on the other it appears more elongated, and again it is common to meet with distorted and swollen, vacuolated forms, which are interpreted as involution or degeneration forms. These latter are especially numerous in older cases and old cultures.

On gelatin and agar containing 2.5 to 3.5 per cent. of salt and in bouillon a fairly characteristic growth results. In the case of the agar involution forms are obtained, among which long, slender

bacilla, which are segmented and present a vacuolated appearance, are especially noteworthy. In this state they stain quite badly and have lost a certain degree of their virulence. In bouillon the organism often forms long chains of well-rounded bodies which are quite similar to a coccus. During its growth in bouillon it forms flakes or flocculi, which rapidly sink to the bottom of the tube, leaving the liquid clear above. Stalactite or stalagmite formations may also be seen, starting from the walls of the tubes or from suspended droplets of oil or butter. Colonies on gelatin about thirty-six hours old are warty, strongly refractive formations, which often present a delicate, irregularly indented margin. Even after twenty-four hours one can obtain smears in which 50 to 100 bacilli are grouped in little colonies of irregular form, while examination of the plates with a magnifying power of 60 diameters reveals scarcely any growth. The organism does not liquefy gelatin. The optimum temperature for growth is between 25° and 30° C.

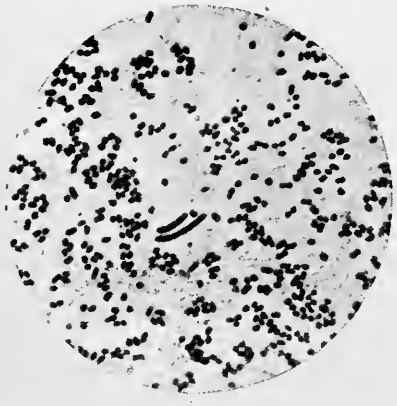


FIG. 204.—Plague bacilli from agar culture. × 1100 diameters. (Park.)

For staining purposes, borax methylene blue (a solution of 2 per cent. methylene blue in 5 per cent. borax water) or Löffler's alkaline methylene blue may be conveniently employed. In the first instance we stain for one-half minute, in the second for two or three minutes. The polar staining is in this manner quite satisfactory. The organism is decolorized by Gram.

In advanced cases of bubonic septicemia the specific organism may be found in the blood in small numbers. Toward the end of rapidly fatal cases they become more numerous, and may then be demonstrable directly with the microscope. According to Bell the bacilli can be found in all cases and at all stages of the disease by using Ross' dehemoglobinizing method (p. 134).

In cases of the pneumonic type of the disease the bacillus occurs in the sputum in enormous numbers. By direct observation, however, it may not be recognized immediately, and it is best in every case to resort to culture as well. The organism may be found in the sputum on the first day of the disease.

Vincent's Spirilla and Fusiform Bacilli.—In cases of Vincent's angina (ulceromembranous angina and stomatitis) smears from the exudate will be seen to contain organisms which are essentially of two types,

viz., spirilla and long fusiform bacilli (Fig. 205). Occasionally, though exceptionally, the bacilli only may be found. The spirilla usually present three or four convolutions and are generally actively motile. They measure from 36 μ to 40 μ in length by 0.5 μ in breadth. The bacilli measure from 6 μ to 12 μ in length and are somewhat stouter in the middle than at the ends; not infrequently they appear vacuolated. They may occur in twos, joined end to end, and usually scattered uniformly throughout the preparation. They are nonmotile. Spirilla and bacilli are readily stained with a dilute solution of carbol fuchsin (1 to 20), which should be filtered before use. Löffler's blue and gentian-aniline water may likewise be used. The bacilli are obligate anaërobes; the spirilla may be obtained together with the bacilli in mixed cultures on Noguchi's spirochetal medium (p. 526).

FIG. 205.—Spirilla and fusiform bacilli of Vincent's angina.

Of late the opinion has been expressed that the spirilla and bacilli may represent stages in the life history of a trypanosome.

Both organisms have occasionally been found associated with diphtheria bacilli.

Micrococcus Catarrhalis.—This organism is frequently seen in the sputa and nasal discharge. It is larger than the common staphylococci, but, like these, frequently occurs in lateral pairs, the contiguous sides being concave.

Micrococcus Tetragenus.—This organism is frequently seen in the sputum under the most varied pathological conditions and may also occur in the mouths of perfectly healthy individuals. It is a coccus occurring in fours, each measuring about 1 μ in diameter. The form which is found under normal conditions, in contradistinction to that seen in disease, cannot be cultivated.

The Bacillus of Glanders.—In glanders the specific bacillus is frequently present in the blood and may be demonstrated by staining dried preparations for five minutes with a concentrated alcoholic solution of methylene blue mixed with an equal volume of a 1 to 10,000 solution of potassium hydrate just before using. From this mixture the specimen is passed for a second or two into a 1 per cent. solution of acetic acid which has been tinged a faint yellow by the addition of a little tropeolin 00 solution; it is then decolorized by washing in water containing 2 drops of concentrated sulphuric acid and 1 drop

of a 5 per cent. solution of oxalic acid for each 10 c.c. In specimens thus stained the bacilli appear as short rods measuring from 2μ to 3μ in length by 0.3μ to 0.4μ in breadth, often containing a spore at one end.

The Anthrax Bacillus.—The anthrax bacillus is an organism which measures from 5 to 12μ in length by 1μ in breadth, with square ends (Fig. 206). When grown on artificial media it usually forms long threads in which the individual segments are distinctly worked off. Under unfavorable conditions of nutrition it forms spores, one of which may be seen in each segment; in the tissues this never occurs, but capsule formation then takes place. It grows readily on the ordinary media at a temperature of from 18° to 43° C., the caput medussæ-like growth on gelatin or on agar being especially charac-

FIG. 206.—Anthrax bacillus. × 900 diameters. Agar culture. (Park.)

teristic (Fig. 207). It can easily be stained with the usual anilin dyes. Good results are obtained by stirring for 5 to 10 minutes in a mixture of 30 c.c. of a concentrated alcoholic solution of methylene blue and 100 c.c. of a 1 to 10,000 solution of sodium hydrate, after which they are washed for 5 to 10 seconds in 0.5 per cent. acetic acid, then with alcohol, and finally dried. When present in large numbers it is not necessary to stain the blood, as the organism can then be seen without difficulty in the wet specimen.

In doubtful cases, in which a microscopic examination of the blood yields negative results, a few cubic centimeters may be injected into a mouse or a guinea-pig in the blood of which the bacilli will soon be found in enormous numbers if the disease is anthrax.

McFadyean has described a color reaction of anthrax blood which seems to be pathognomonic of the disease. Smears are prepared as usual and, when air-dry, fixed by heat—until the slide has become a

little too hot to be held against the skin. On cooling, the specimens are stained for a few seconds with a 1 per cent. aqueous solution of methylene blue (medicinal of Merck), or with one of Grübler's methylene blues, modified by boiling with 0.5 per cent. of sodium bicarbonate. After washing with distilled water they are dried with filter paper, subsequently by heat, and mounted in balsam. Anthrax blood then shows a distinct reddish or purplish tone, especially when held against the light, while other blood appears pure blue or greenish blue.

FIG. 207.—Colonies of Bacillus anthracis upon gelatin plates: *a*, at the end of twenty-four hours; *b*, at the end of forty-eight hours. × 80. (F. Flügge.)

Microscopic examination of the amorphous intercellular material shows the same result.

According to Heim, who has described the same reaction independently of McFadyean, the color change is due to mucin derived from the capsules of the bacteria.

PART II

THE ESSENTIAL FACTORS IN THE LABORATORY DIAGNOSIS OF VARIOUS DISEASES

ACHYLIA GASTRICA (NON-MALIGNANT)

Essential Factors.—Irregular anemia of considerable severity; persistent absence of hydrochloric acid, ferments, and zymogens; absence of occult bleeding; normal urinary picture.

The Blood.—*The Red Cells and Hemoglobin.*—In some cases of achylia gastrica anemia is one of the most prominent features of the disease; occasionally it is indeed the only factor which attracts attention. In others it is absent or insignificant. The corpuscular diminution in cases belonging to the first class is frequently so extensive as to suggest the existence of pernicious anemia and in former years "gastric atrophy" was regarded as a possible etiological factor of the disease. As Einhorn very properly suggests, however, it is more likely that both conditions may have a common cause, or that achylia furnishes a favorable basis for the development of pernicious anemia. Both no doubt may co-exist, but we now know that the severe anemia which is usually seen in achylia differs in several important respects from true pernicious anemia. In Einhorn's series of 15 cases a diminution in the number of the red cells was only noted in four, the two lowest counts being 1,536,000 and 2,600,000. Regarding the color index, I have not been able to find any data, but I assume from the fact that no macrocytosis was observed that it was not increased. Poikilocytosis, on the other hand, may be marked. Nucleated red cells were absent in Einhorn's cases.

The Leukocytes.—Regarding the leukocytes, I have not been able to find any satisfactory data.

The Gastric Contents.—Vomiting occurs in some of the cases. Occasionally it takes place very soon after the ingestion of a meal, while in others a variable length of time elapses. The material is practically undigested. Hematemesis has not been observed.

The diagnosis of the disease can only be made by repeated examination of the stomach contents after the administration of a test

meal. Single observations are of little if any value. The amount of material which may be obtained one hour after Ewald's test breakfast is relatively small, which, according to Einhorn, is owing to the lack of gastric secretion and the greater rapidity with which the fluids of the test meal pass through the pylorus. The bread shows no evidence of digestion. Free hydrochloric acid is absent and the total acidity also is very much diminished—viz., to 1 to 4; it is rarely higher. A neutral reaction, however, is exceptional. Lactic acid is usually absent or present only in traces; larger amounts are exceptional, and practically only met with when marked dilatation complicates the case. The ferments and their zymogenes are likewise absent and the reactions for albumoses and peptone consequently negative or minimal.

In extreme cases the production of mucus also may be arrested, but in the earlier stages particularly a fair amount of viscid mucus is frequently found and may at times be demonstrable through the entire course of the disease.

The motor power is usually good, as evidenced by the fact that the stomach is found empty four or five hours after a test dinner, and similar findings early in the morning. Exceptionally, when atrophy of the muscular coat complicates the condition, marked atony and dilatation may, of course, develop with consequent retention of food material.

Not infrequently small pieces of mucous membrane are found in the washings, and in these, as Einhorn has shown, normal glands may still be demonstrable, in spite of the achylia.

Achylia and anadeny are hence not interchangeable terms.

The Feces.—In some of the cases diarrhea exists and may control the clinical picture, while, as a rule, there is a moderate tendency to constipation. Occult bleeding, according to Schloss, and in contradistinction to Kuttner, is rare.

The Urine.—In a number of cases of achylia gastrica, with and without anemic manifestations, Strauss found practically normal conditions. The ammonia content was not increased and an estimation of the chlorides, phosphoric acid, conjugate sulphates and toxicity showed no deviations from the normal; the same held good for the uric acid values, unless the condition was complicated with pernicious anemia.

ACROMEGALY

Essential Factors.—Variable blood picture; marked tendency to diabetes.

The Blood.—Da Costa gives the blood findings in two cases of acromegaly. In the one the red count, hemoglobin, and color index were 4,620,000, 86 per cent., and 0.93, and in the other, 2,880,000, 60 per cent., and 1.04 respectively. The leukocytes in the first case

numbered 8000, of which, 31.7 per cent. were small mononuclears, 2.1 large mononuclears, and 66.2 polynuclear neutrophiles, while in the second case the total number was 4890, of which, 21 per cent. were small mononuclears, 7 large mononuclears, 71 polynuclear neutrophiles, and 1 per cent. eosinophiles. Coagulation, fibrin formation, and the number of plaques were normal; nucleated red cells were absent.

Sabrazés and Bonnes give the following results in two additional cases:

	Case I.		Case II.	
Hemoglobin	78.0	per cent.	112.0	per cent.
Red cells	4,960,000		5,874,500	
Color index	0.78	"	0.95	"
Leukocytes	11,780		2480	
Small mononuclears	41.9	"	45.0	"
Large mononuclears	7.3	"	3.3	"
Polynuclear neutrophiles	45.7	"	42.0	"
Eosinophiles	2.6	"	6.5	"
Mast cells	....		0.7	"
Blood platelets	....		57,040	

In three cases of acromegaly, on a purin-free diet, Falta and Nowaczynski found the endogenous uric acid to be twice the normal average or greater, while the administration of sodium nucleinate gave rise to a marked increase. This in contradistinction to two cases of dystrophia adiposogenitalis associated with hypophyseal tumor, in which the endogenous output was normal or subnormal, while the administration of sodium nucleinate caused only a slight increase. The writers suggest that the behavior of the endogenous output may be of some moment in the differential diagnosis of acromegaly.

The Urine.—The only material abnormality in the urinary picture is the manifest tendency to diabetes. Von Noorden mentions that of five cases which he saw himself, four were diabetic. In two of these the clinical picture differed in no way from ordinary diabetes, while in the two others the glycosuria underwent temporary changes which were not dependent upon the nature of the food, suggesting the action of independent (neurogenic) factors.

ACTINOMYCOSIS

Essential Factors.—Marked chlorotic anemia; hyperleukocytosis; presence of "sulphur" granules in the sputum or pus of the corresponding abscesses; tendency to amyloid degeneration of the kidneys and consequent albuminuria.

The Blood.—In moderately advanced cases of actinomycosis a well-pronounced *chlorotic anemia* is a common symptom; as the case progresses this may become quite intense, brought about in part, no doubt, by associated pyogenic infections. In one case mentioned

by Schmidt the red count fell to 2,550,000 and the hemoglobin to 23 per cent. (color index, 0.45); in another instance (Da Costa) the red count was 4,985,000 and the hemoglobin 55 (index, 0.55).

The Leukocytes.—The leukocytes are apparently increased in all cases. In a series of 11 cases (Schmidt, Da Costa, Ewing, Erving, and Cabot) the counts ranged from 10,000 to 36,200. The higher values are more apt to be found when the disease attacks the deeper structures (lung and liver) than in connection with superficial lesions. In Da Costa's case, with 12,000 leukocytes, the differential count showed 25.5 per cent. of small mononuclears, 7.3 of large mononuclears, 60 per cent. of neutrophiles, 2.4 eosinophiles, 0.6 mast cells, and 2.4 myelocytes. The plaques were much increased.

The Sputum.—In cases of actinomycosis of the lung the sputum may present an appearance that is usually seen in bronchitis, while in other cases it is more purulent and in isolated instances it has been described as rusty. The diagnosis depends upon the demonstration of the actinomycotic sulphur granules (for a description of which see the general section on Sputum). The number of these is variable; sometimes they are scanty, while at others the patient may himself call attention to the peculiar appearance of the sputum. Occasionally elastic tissue may be found.

The Pus.—The pus which is obtained from actinomycotic abscesses, particularly in the earlier stages of the disease, represents a whitish or, in consequence of hemorrhages, a dirty brownish material, in which the sulphur granules can be demonstrated on careful search.

The Feces.—When the disease affects the intestines corresponding findings may be made in the feces.

The Urine.—The urine presents no special abnormalities during the earlier stages of the disease, but in cases where extensive suppuration has gone on for a long time amyloid disease of the liver, intestines, and kidneys may develop with consequent changes in the urine. Albumin is then quite abundant, while tube casts and corpuscular elements are relatively scanty. Quite suggestive are abrupt and frequent changes in the content of albumin, as also in the amount of urine, which usually is copious and light colored.

ACUTE YELLOW ATROPHY (ICTERUS GRAVIS).

Essential Factors.—Normal red count; moderate hyperleukocytosis, cholemia and urobilinemia; vomiting and diarrhea; hematemesis; low urea values; high ammonia values due to acidosis; presence of leucin and tyrosin in the urine; albuminuria and cylindruria.

The Blood.—Unfortunately the available data are too meager to construct an adequate blood picture of acute yellow atrophy. From the isolated reports it appears that there is a normal number of the red cells and a moderate increase of the leukocytes, with no special

changes in the differential count. Emerson mentions a case with 4,800,000 red cells and 12,700 leukocytes; Grawitz cites one with 5,150,000 and 12,000, and Cabot one with 5,520,000 and 16,000 respectively. The hemoglobin is moderately reduced—60 to 80 per cent.

The serum is markedly tinged with bile or urobilin.

Gastro-intestinal Tract.—Vomiting and diarrhea are common symptoms, and not infrequently there is hematemesis and the discharge of blood in the stools. When this is absent the feces will be found to be light colored, owing to a deficiency in the biliary secretion.

The Urine.—The amount of urine is usually much reduced, while the specific gravity is increased. Bile pigment and urobilin are present in large amount. Especially noteworthy is the great decrease in the amount of urea which is noted in some of the cases. Frerichs states that in extreme cases it may be reduced to traces or even be absent. This, however, is not necessarily the case, for in some instances very fair quantities have been noted. Formerly this deficiency was interpreted as indicating a functional insufficiency of the liver, but it is noteworthy that there is usually no material increase in the elimination of nitrogen in other forms. The inference thus seems justifiable that the low urea values are referable to the ingestion of correspondingly small amounts of food. This assumption is not invalidated by the moderate increase in the ammonia nitrogen, which finds a legitimate explanation in the associated acidosis.

In five fatal cases mentioned by v. Noorden the urea nitrogen ranged between 52.4 and 81.1 per cent., and the ammonia-nitrogen between 4.7 and 37 per cent. When fixed alkalies are administered at a time when the latter values are increased, a material decrease results. The actual acid factors which enter into consideration are only in part known: Schultzen and Riess have demonstrated the presence of sarcolactic acid and oxy-amygdalic acid; other observers have found the fatty acids increased; Senator and Soetbeer have reported the presence of diacetic acid. As a result of autodigestion of the liver, leucin and tyrosin are liberated in large amount and may appear as such in the urine; the amount which is thus excreted is, however, quite variable; sometimes traces only are found, even though the liver, post mortem, can be shown to contain large quantities.

In some instances digestive glucosuria has been observed.

Albumin is usually present in small amounts, and with it hyaline, granular, fatty, and epithelial casts.

ADDISON'S DISEASE

Essential Factors.—Hyperglobulism giving place to corpuscular anemia; methemoglobinemia; irregular leukocytosis. No uniform urinary changes.

The Blood.—*The Red Corpuscles and Hemoglobin.*—In the majority of cases, when the disease is well advanced severe anemia is the rule (as low as 613,000), while in the early stages normal values are found. A few writers mention the occasional occurrence of polycythemia with high hemoglobin values. Cabot thus speaks of one patient with 6,240,000 red cells and 90 per cent. of hemoglobin. Acuna mentions another in which the hyperglobulism (5,000,000 to 8,000,000) did not disappear until late in the disease, and Neumann records a count of 7,700,000. In the anemic cases the loss of hemoglobin equals or exceeds that of the red cells; the reverse is uncommon. Colat reports an index of 3.9.

Of special interest is the observation of Tschirkoff that *methemoglobin* occurs in some of the advanced cases, while, according to the same writer, in some of the earlier cases there is a decided increase of the reduced hemoglobin, which may even exceed the oxyhemoglobin.

Nucleated red cells are only exceptionally seen; polychromatophilia is more common; stiple cells are rare.

The specific gravity is normal (1.055 to 1.056).

Treatment with suprarenal extract may bring about a marked improvement in the condition of the blood.

The Leukocytes.—The number of the leukocytes is variable, but usually normal; in some cases a notable leukopenia has been noted and *sub finem vitæ* a moderate hyperleukocytosis (13,000). The differential count may reveal a moderate lymphocytosis (28 to 36 per cent.), a moderate increase of the eosinophiles (6 to 8 per cent.), and the presence of a few myelocytes has also been noted.

The Urine.—The urine shows no characteristic changes. The quantity is generally normal, in some cases diminished, in others increased. Urobilin may be present in excess; melanin has been at times observed. The indican is commonly increased. Albumin is usually absent. The volatile fatty acids are much increased. Kreatinin is diminished and urea about normal.

ADIPOSITY (SEE OBESITY)

AMEBIASIS (AMEBIC DYSENTERY AND AMEBIC LIVER ABSCESS)

Essential Factors.—Secondary anemia; hyperleukocytosis of the neutrophilic type with normal eosinophile values; presence of the Amœba dysenteriæ in the feces and in the pus of the corresponding liver and lung abscesses; no special urinary features.

The Blood.—*The Red Cells and Hemoglobin.*—On counting the red corpuscles in cases of amebic dysentery one is often surprised to find the resultant figure so manifestly out of proportion to the otherwise self-evident anemia of the patient. This is owing to the existence of a relative polycythemia in consequence of a concentra-

tion of the blood, brought about by diminished ingestion and relatively excessive elimination of fluids. In Futcher's series of 38 uncomplicated cases of amebic dysentery the average count was 4,802,000, with 63 per cent. of hemoglobin. Very nearly the same figures were obtained in 15 cases complicated with liver abscess, viz., 4,250,000 and 66 per cent. The lowest count in the Hopkins series was 2,200,000.

The Leukocytes.—The leukocytes are increased in a considerable proportion of the dysentery cases, but the average increase is but little above the maximal normal, viz , 10,600. In ten of Futcher's series the count was above 18,000, however, and in two there were 40,000 and 47,000 respectively. In view of these extensive fluctuations it is of little interest from the standpoint of diagnosis that the average count in the abscess cases is 7750 above the average in the uncomplicated cases of amebic dysentery. As Futcher has pointed out, there are many abscess cases in which the count is lower than what is seen in many uncomplicated cases of dysentery. His average in the abscess cases was 18,350; in two the count was below 10,000, while the highest value was 53,000. The leukocytosis is of the neutrophilic type, but according to McCrae the eosinophiles are present in practically normal percentages. Considering the existence of a hyperleukocytosis this would really mean an absolute hypereosinophilia. This was also found by Amberg in amebic dysentery occurring in young children.

The Pus.—In suspected cases of amebic abscess the liver should be carefully explored with the needle. The pus which may thus be obtained is brownish red in portions and has been likened to anchovy sauce in general appearance. On microscopic examination innumerable pus cells are found in various stages of degeneration, some red blood corpuscles, amorphous pigment derived from degenerated red cells, rarely well-preserved liver cells, occasionally pieces of necrotic tissue, and most important of all, of course, the Amœba dysenteriæ. The number of amebæ is variable, but usually quite large; they are actively motile, when freshly observed on the warmed slide, and frequently contain red blood corpuscles in their interior. Cultures from the pus are probably always sterile. I am not aware of a single instance at least in which bacteria have been cultivated from an amebic liver abscess.

Perforation of a liver abscess takes place in a large percentage of cases. In Cyr's series of 563 cases it occurred in 159, *i. e.*, in 28.2 per cent.; in 59 of these it ruptured into the lung, in 31 into the pleura, in 13 into the intestine, in 8 into the stomach, and in 2 into the kidney.

Sputum.—When rupture takes place into the lung the diagnosis is often possible from the naked eye appearance of the expectoration, which, like the pus directly obtained from the liver, has been likened to anchovy sauce. At other times it may be tinged a bright yellow

from the presence of bile, and the patient may complain of its bitter taste. When the rupture occurs the pus may pour into the lung with such rapidity and in so large a quantity that the patient may suffocate. More often, however, the abscess empties itself gradually. After perforation has once taken place an abundant discharge may continue for a long time, frequently amounting to 250 to 500 c.c. in the twenty-four hours. The material is then less characteristic; it is essentially mucopurulent, but from time to time a mouthful may be expectorated which still presents the characteristic brownish red appearance that is noted in the beginning. I remember one case of this order which I diagnosticated correctly years ago as a young assistant at the Hopkins. I saw the patient expectorate into the sink in the dispensary, and immediately took him to the ward so that I might have the pleasure of being the first one to show Dr. Osler a case of this kind on his return from Europe. This patient had had dysentery fully six months before, but had completely recovered from it. The search for amebæ in the expectoration was most laborious, but after an hour or longer I found actively crawling specimens which I was able to show. In more recent cases they may be fairly numerous. Elastic tissue is always present and may be found in large amount. In addition, there are, of course, innumerable pus cells, very rarely well-preserved liver cells, but usually some red corpuscles and hematoidin either in crystalline or amorphous form.

When rupture has taken place into the pleura the diagnosis may possibly be made by paracentesis before the material empties through the lung, which probably always occurs sooner or later. I have never seen this accomplished, however.

The Feces.—In the dysentery cases, during the most active stage of the disease the number of stools may vary from 6 to 20 or even 30 in the twenty-four hours. They may be altogether mucoid and only streaked here and there with a little blood-tinged pus. Others seem to be made up of a greenish, pultaceous material in which large, irregular sloughs may be observed. Stools of this order are usually small in amount. Occasionally large brownish liquid evacuations occur, in which particles of purulent material may be seen, often adhering to or embedded in blood-tinged mucus. On microscopic examination one finds pus corpuscles and red cells in greater or less abundance, more or less degenerated epithelial cells, innumerable bacteria, at times shreds of necrotic tissue, and not infrequently Charcot-Læyden crystals. The most important component, of course, is the Amœba dysenteriæ. In fresh stools which have not been exposed to the cold actively moving organisms may be found in variable number, frequently containing red corpuscles. Formerly it was the custom to search for the ameba in bits of hemorrhagic mucopus, but Musgrave and Clegg have pointed out that in order to get the best result the patient should always be given a saline

cathartic, and that the examination should be made from the fluid portion of the stools. The chances of finding the organisms are thus greatly increased, as the washings of the entire colon are brought down in this manner. When the amebæ for any reason are quiescent the diagnosis should only be made with great reserve, as it has repeatedly happened even to experienced workers that swollen epithelial cells have been mistaken for amebæ. If such doubtful cells contain red corpuscles the probabilities are great that they are amebæ, but absolute certainty can only be felt if they move.

After the acute stage has been passed the stools of the patient may resume their usual appearance, so far as naked eye examination goes, excepting, perhaps, the occasional presence of mucus in greater or less amount. On microscopic examination, however, amebæ may be found in some cases even then, and may actually be present in large numbers. In the case referred to above, where the original attack of dysentery had disappeared, but in which an amebic liver abscess had perforated into the lung, and in which I had found amebæ in the sputum only after a long search, it was possible to demonstrate a dozen in a single field ($\frac{1}{6}$ objective), in the mucous material obtained by rectal tube, or covering an otherwise normal looking stool.

Even after the stools have apparently resumed their normal condition acute exacerbations of the dysenteric process may occur from time to time. I would therefore emphasize the importance of a careful search for amebæ whenever a history of a relatively recent diarrhea can be elicited. I remember a great blunder which was thus committed not many years ago, when acute exacerbations of an old dysenteric process had been looked upon as "bleeding piles" and treated accordingly.

So far as the question is concerned whether or not the intestinal tract may become infected with non-pathogenic amebæ, Musgrave and Clegg have come to the conclusion that all amebæ are or may become pathogenic. They emphasize that this proposition, pending a complete solution of the problem, is the only safe one to adopt from the standpoint of public health in the tropics, and that in such countries the appearance of amebæ in the stools should be sufficient grounds for the institution of therapeutic measures, regardless of the nature of the clinical symptoms.

The Urine.—The urinary picture shows no features which are in any way characteristic of amebic infection. So long as dysentery exists there will naturally be oliguria with the concomitant factors: high specific gravity, marked acidity, and tendency to the deposition of urates. In very severe cases mild albuminuria and hyaline cylindruria may be observed. In the abscess cases there are no further manifest deviations; glucosuria in particular is not a feature of the condition. In those very rare cases in which the abscess ruptures

into the kidney the sudden appearance of the pus would naturally attract attention. In a relatively small number of cases there is jaundice and hence choluria.

AMEBIASIS OTHER THAN INTESTINAL OR HEPATIC

The occurrence of amebiasis other than intestinal or hepatic has been repeatedly described, but the identification of the organism has not always been satisfactory. Miura mentions the presence of amebæ in the ascitic fluid of a woman suffering from an abdominal tumor; it is interesting to note that in this case they were also observed in the bloody, mucous stools. Celli and Fiocca "cultivated" amebæ from the larynx of a case of tubercular laryngitis, from the lungs in 10 cases of tuberculosis, in 6 cases of pneumonia, in 15 of bronchial catarrh, three out of fifteen times from the female urinary tract, and once from the stomach of an infant. Kartulis found amebæ in the necrotic bone of the lower jaw, Flexner, in an abscess located in the floor of the mouth, and in a gangrenous surgical wound, in a case of liver abscess.

Leyden and Schaudinn met with ameboid bodies in the aspirated ascitic fluid of two cases of abdominal tumor, and Baelz found them in the bladder and vagina of a young woman who had had hemorrhagic cystitis and later died of pulmonary and genital tuberculosis. Jürgens found them in the urine of a patient suffering from a tumor of the bladder, and Wijuhoff likewise found them in the urine in four cases. Posner discovered amebæ, some of them containing red blood cells, in the bloody urine of a patient who had never been out of Berlin.[1]

ANEMIA INFANTUM PSEUDOLEUKEMICA (v. JAKSCH'S ANEMIA)

Under the above name, v. Jaksch has described a type of infantile anemia which is usually observed during the first year of life, more rarely in the second or third, which is characterized clinically by enlargement of the spleen and multiple enlargement of the lymph glands, while examination of the blood shows a marked diminution of the specific gravity, and considerable loss of red cells and hemoglobin, poikilocytosis, presence of nucleated red cells, and hyperleukocytosis, usually of the polynuclear neutrophilic type, less commonly of the lymphocytic variety.

Subsequent investigations have rendered it very probable that v. Jaksch's anemia is not a disease *sui generis*, but merely a manifestation of rickets, congenital syphilis, or some other underlying pathological condition.

[1] The above summary of extra-intestinal, sc. hepatic amebiasis is taken from Musgrave and Clegg's monograph.

ANEMIA (POSTHEMORRHAGIC)

Essential Factors.—Secondary anemia with at first normal and subsequently diminished color index; presence of normoblasts; neutrophilic hyperleukocytosis; increase of plaques.

The Blood.—*The Red Cells and Hemoglobin.*—The blood picture in posthemorrhagic anemia, so far as the red cells and hemoglobin are concerned, will largely depend upon the time at which the examination is made, and upon the nature of the underlying pathological conditions. When the hemorrhage is of traumatic origin and occurs in a previously healthy individual the purest type of secondary anemia will be encountered. There is then a true oligemia, which at first affects both the corpuscular elements and the plasma, the result being that a red count and hemoblobin estimation at this time reveals no loss of either. The number of corpuscles pro volume of blood and the amount of hemoglobin per cell is exactly what it was before the hemorrhage. As soon as the volume of fluid in the bloodvessels has been restored to the original bulk, however, the actual anemia becomes at once apparent, both in the red count and in the hemoglobin content. This usually occurs within a few hours after the hemorrhage, and is hastened by the introduction of liquid from without. The color index at that time is perfectly normal. Within the next days, however, there is a further loss of red cells and hemoglobin, which is usually referred to a lack of resistance on the part of the young cells which are at first sent out from the bone marrow and which themselves are not as rich in hemoglobin as the normal circulating erythrocytes. The color index then accordingly tends to be subnormal. Subsequently, as properly matured red cells appear, the index again rises.

The actual count in posthemorrhagic cases will thus depend to a great extent upon the amount of blood that is lost and the time at which the examination is made. The lowest counts are usually met with between the second and the eleventh day. In Rieder s cases the figures varied between 1,300,000 and 3,335,000, and in those of Strauss and Rohnstein between 1,119,000 and 4,420,000. A sudden reduction in the number to 1,000,000 or less is usually followed by a fatal result. Exceptions, however, occur. Hayem thus cites a case of postpartum hemorrhage where the patient recovered in spite of a reduction of the red cells to 11 per cent. of the normal.

A restoration to normal conditions, so far as the red cells go, usually occurs in from three to four weeks, unless the hemorrhage has been exceptionally severe. The color index, however, is apt to remain below normal for some time yet, so that a certain degree of chlorotic anemia continues into the convalescent state.

A small number of normoblasts may be found in almost every case after the second or third day following the hemorrhage, until normal

relations have been restored; but their search is sometimes a long one. More rarely there is a sudden influx of nucleated red cells, a state of affairs which v. Noorden has designated as a *blood crisis*. This may be preceded and accompanied by a very extensive increase of the leukocytes. Ehrlich cites an instance of this kind, originally reported by v. Noorden, where the normoblasts were so numerous, while hyperleukocytosis of high grade existed at the same time, that at first sight the blood condition suggested the existence of a myeloid leukemia. The increase of the red cells in this case amounted to almost double the original number.

Megaloblasts are rarely seen, and play no role in normal blood regeneration. A certain degree of polychromatophilia, however, is not uncommon, and occasionally one may meet with isolated stiple cells (so-called granular degeneration).

The Leukocytes.—In almost all cases there is a well-pronounced posthemorrhagic leukocytosis of the neutrophilic type which appears quite early—usually within a few hours—and persists for several days. Generally speaking, the degree of increase is proportionate to the amount of blood that is lost and the regenerative power of the individual. Only in exceptionally severe cases is it lacking. Rieder noted an increase to 15,000 after a pulmonary hemorrhage, 32,600 after a hemorrhage due to uterine cancer, and 26,500 after a hemorrhage referable to gastric ulcer.

The Plaques.—The plaques are markedly increased.

The Urine.—The urine shows no special abnormalities. Occasionally there is slight albuminuria.

ANTHRACOSIS

To some extent particles of carbon may be found in the sputum of almost every individual. The expectoration in such cases is of a pearl-gray color, and is brought up in larger or smaller masses, especially in the morning upon rising. Larger amounts are noted in miners and in those who are brought into close contact with coal dust. Microscopically, particles of carbon and epithelial cells, of the alveolar type, as well as leukocytes loaded with the pigment, are seen.

ANTHRAX

Essential Factors.—Presence of the anthrax bacillus in the blood.

The Blood.—Adequate data from which a blood picture of anthrax could be constructed are wanting.

In infections of some of the lower animals the corresponding organism may be demonstrated in the blood in large numbers. In man this is apparently possible only in exceptional instances, late in the disease, when general septicemia has developed, and even then

not in all cases. Blumer and Young have reported a case in which they were able to obtain the organism by culture, and to demonstrate it in the blood smears directly. If in a suspected case negative results are obtained, a couple of cubic centimeters of blood may be injected into a mouse or a guinea-pig, in the blood of which the bacilli will soon be found in enormous numbers, if the disease be anthrax.

In addition, the color reaction described independently by McFadyean and Heim may be tried. (See bacillus of Anthrax.)

The Pus.—In the pus obtained from anthrax pustules the organism may be demonstrated directly, as well as by culture.

The Urine.—Regarding the urinary picture there are no available data.

APPENDICITIS

Essential Factors.—Hyperleukocytosis; increase of neutrophiles; decrease or absence of eosinophiles.

The Blood.—*The Red Cells and Hemoglobin.*—These show no material deviation from the normal in ordinary cases, while in severe cases, or where chronic suppuration occurs, a certain degree of secondary anemia will of necessity develop.

The Leukocytes.—An increase in the number of the leukocytes (10,000 to 30,000) is noted in all cases of active appendicitis at some period of the attack, and, generally speaking, runs a course parallel to the intensity of the infection. The higher the count the greater is the probability of the existence of a purulent condition. This, however, is not an invariable rule, and it is noteworthy that there is frequently a remarkable discrepancy between the height of the leukocytosis and the extent of the inflammatory involvement. In children especially it is quite common to find the leukocytes markedly increased (20,000 or more), with relatively little manifest disease at the time of the operation. It is better, therefore, to look upon the degree of leukocytosis rather as a sign of the degree of systemic reaction than as evidence of the intensity of the infection, although the curve of the two conditions will in the nature of things frequently coincide. If we interpret the leukocytosis as evidence of the defensive reaction of the body, we can also understand why it is that in exceptionally severe cases, or upon the development of general peritonitis, the leukocytosis may be relatively little marked (8000 to 12,000) or absent altogether, the defensive mechanism being overwhelmed by the intensity of the toxemia.

An increasing leukocytosis during the progress of the disease indicates in a general way that the morbid process is continuing and probably becoming more active. A decreasing leukocytosis, on the other hand, may indicate either that the condition is improving or growing very much worse. The interpretation of what is actually taking place is very much aided by the differential count.

This, indeed, is indicated in every case in which acute appendicitis is suspected. It affords information which the absolute count cannot furnish, and I would emphasize that in the diagnosis of septic conditions (of which appendicitis, of course, is a typical example) no laboratory examination is more important than it. For years past I have insisted upon the recognition of the *septic factor*—meaning thereby an increase of the neutrophiles, when associated with a decrease or absence of the eosinophiles—as one of the most important factors in the diagnosis of appendicitis, and one which is present as long as the disease is active, irrespective of the absolute count. It is met with as soon as there are clinical symptoms indicating inflammatory disturbance and persists until the attack has come to an end. It is never absent when a developing peritonitis causes the total leukocyte count to drop, and will thus prevent one of the most serious errors into which the absolute count, taken by itself, may lead the physician. Its importance cannot be overestimated, and it is sincerely to be hoped that physicians at large shall resort to it in doubtful cases with the same constancy with which they take the patient's pulse and temperature. In the diagnosis of acute appendicitis it furnishes more valuable information than either; it serves to differentiate sharply what is unquestionably the most important acute abdominal disease, in which alert attention and prompt action are called for, from all those vague abdominal disturbances in which abdominal pain plays a signal role, but in which a relatively unimportant condition exists. For years past I have told my students that they can rest in peace when acute abdominal pain is associated with a lymphocytosis, but that keen attention is necessary if the differential count reveals the septic factor. Exceptions to this rule are very rare, and so far as my own observations are concerned, limited to the accidental coincidence of an appendicitis with the presence of those metazoic parasites which are apt to cause a hypereosinophilia. In one case of trichinosis which was complicated by appendicitis the polynuclear neutrophiles were markedly increased, while the eosinophiles were present in maximal normal numbers. In several cases of oxyuris appendicitis which I have had occasion to observe the eosinophiles were absent or at least markedly diminished. Cecil and Bulkley report 21 cases of oxyuris appendicitis and 2 of trichocephalus appendicitis, all but one of which were catarrhal. They remark that the eosinophiles were counted in only a very few, but that in these the percentage was "well within the normal limits," excepting in one child, whose appendix contained a few oxyurids and in whose stools the ova of trichocephalus was found. In this case the eosinophiles numbered 44 per cent.

In young children, however, one should bear in mind that the polynuclear neutrophiles are even normally much less numerous than in adults, and that the figures obtained in a given case must be com-

pared with what is normal for that age (see p. 57). Whereas in the adult we would expect a value between 80 and 85 per cent. in the average case of acute appendicitis, a count of 50 to 55 per cent. in a child of eighteen months should be viewed in the same light. Unless this is borne in mind some grave mistakes will occur. But here as there low eosinophile values, or the absence of these cells, is constant.

In a case of appendicular abscess in a baby, eleven months old, occurring in the practice of Dr. L. P. Hamburger, of Baltimore, the small lymphocytes numbered 34 per cent., the large 11.6, the neutrophiles 54, while no eosinophiles were seen while counting 300 cells.

While hyperleukocytosis and the septic factor may thus be viewed as practically a constant symptom of appendicitis at some time during the course of the disease, it must not be forgotten that both are merely the expression of an inflammatory reaction of a certain type, and that neither is indicative of inflammation of any one organ. The question of appendicitis will hence enter into consideration only, if other symptoms exist which point to disease of the anatomical contents of the right iliac fossa or adjacent regions. Liver abscess, suppurative cholecystitis, cholangitis, pyelonephritis, endometritis, parametritis, oöphoritis, and certain cases of purulent cystitis are thus similarly accompanied by a neutrophilic hyperleukocytosis with a decrease or absence of eosinophiles, in so far, at least, as infection with the common pus organisms is concerned. In typhoidal infections, hyperleukocytosis is only exceptionally seen, and after the first few days it disappears, giving place to a rapidly developing lymphocytosis and splenocytosis, which become more and more pronounced as the disease progresses. Tubercular infection in the early stages of the disease almost always show a lymphocytosis, with frequently maximal eosinophile values, while later on when infection with the common pus organisms is superadded a neutrophilic increase is commonly seen. It is interesting to note, however, that in a case of tuberculosis of the cecum which I was able to observe, the eosinophiles persisted in normal numbers. This association, I would emphasize, is not the septic factor, and should excite suspicion. Similar conditions may be observed in malignant disease.

So far as numerical values are concerned, the most important data are collected in the accompanying table, and are based upon an analysis of 186 cases.

Absolute count.	Septic factor.	Non-purulent.	Purulent cases.
Lower than 5,000	76 to 80 per cent.	6	0
5,000 to 10,000	78 to 84 "	32	8
10,000 to 15,000	80 to 86 "	16	33
15,000 to 20,000	84 to 90 "	6	46
20,000 to 25,000	86 to 92 "	0	24
25,000 to 50,000	86 to 98 "	0	15

In cases in which a well-walled abscess exists at the time when the patient first comes under observation, the total leukocyte count may be normal; it may then remain so, or undergo fluctuations of greater or less degree. In these cases also more information is derived from the differential than from the absolute count; 8000 to 10,000 leukocytes may, after all, mean nothing, while a neutrophilia of 75 per cent. or thereabouts, when accompanied with subnormal eosinophile values, is never observed where septic infection does no exist.

In chronic appendicitis without exudate the blood examination shows nothing normal.

Bloodgood, in an analysis of some of the Hopkins cases, says the following regarding the absolute numerical values in the various types of the disease:

Observed within forty-eight hours the number of white blood cells is in the majority of instances of great value in pointing to the extent of the inflammatory condition of and about the appendix. Cases of recurrent appendicitis or of appendicitis suffering from the first attack, first observed practically at the end of the attack when the clinical symptoms are subsiding, rarely show an increase in the white cells. In a few instances, first observed within forty-eight hours after the beginning of the attack, but when the symptoms are subsiding, there have been a few leukocyte counts of 15,000, which have fallen rapidly within a few hours to 10,000 and 7000. In the cases admitted within forty-eight hours with acute symptoms, if on account of the clinical picture operation has been delayed, a falling leukocytosis has always been observed. These patients have recovered, and at a later operation the appendix was found to be the seat of a diffuse inflammation, but there was no evidence of pus outside the appendix. In one case admitted sixteen hours after the beginning of the attack the leukocytes fell in ten hours from 17,000 to 13,000, and in twenty-four hours to 11,000, associated with disappearance of the symptoms. With one exception, the highest first leukocyte count in this group has been 17,000, falling in a few hours to 12,000, 9000, or even lower. A patient admitted twenty hours after the beginning of the acute attack had a leukocytosis of 22,000; the clinical symptoms, however, were not very marked. The patient was observed eight hours; during this period the leukocytes fell to 16,000 and the local symptoms practically disappeared. Within the succeeding twenty-four hours the leukocytes were 11,000, then 8000, 7000, and 6000. Although this patient with a leukocytosis of 22,000 at the end of twenty hours recovered, and there is every reason to believe that the inflammatory condition about the appendix subsided, nevertheless it is an exception to the general rule, and it would be safer, I believe, to operate in those cases of acute appendicitis observed within the first forty-eight hours with a leukocytosis of 20,000.

In acute diffuse appendicitis with operation and recovery the highest count observed was 25,000 thirty-six hours after the beginning of the attack. At operation in this case intense inflammation and a large amount of exudate were found about the appendix.

In gangrenous appendicitis with operation and recovery the leukocytosis is higher (25,000 to 35,000) and rises more rapidly. As Bloodgood says, the study of the leukocytosis is here of the greatest importance in the early recognition of a grave inflammatory condition of the appendix, which without doubt would lead to general peritonitis and death unless early operation be instituted.

A very high leukocytosis within forty-eight hours after the beginning of the attack is suggestive, but not at all positive, of beginning *peritonitis*. The leukocyte count, however, does not seem to help in such cases with regard to prognosis. After the second day in cases in which the peritonitis has been present longer Bloodgood never has observed recovery with a low leukocyte count. If the leukocytosis remains still high at this period, however, the prognosis seems better for ultimate recovery after operation.

In chronic suppuration the results are less decisive; there are cases, indeed, in which notwithstanding the existence of extensive intraperitoneal accumulations of pus no increase of the leukocytes occurs.

The Urine.—In the majority of cases the urine shows no deviation from the normal. When the appendix dips down into the pelvis pollakiuria is frequent. Albuminuria and hyaline cylindruria may be seen in especially severe cases.

ARTHRITIS DEFORMANS

The Blood.—In uncomplicated cases of arthritis deformans there is neither anemia nor a change in the number of the leukocytes of any notable degree. In Ewing's series of 40 cases the red cells ranged between 4,148,000 and 5,980,000 (average 5,112,000) and the hemoglobin from 80 to 100 per cent. (average 94), while the leukocytes averaged 8885, exceeding 10,000 in only 5 cases. The differential count shows an occasional increase in the number of the small mononuclears.

The Urine.—The urinary picture shows no special abnormalities. Some writers mention a diminished elimination of phosphoric acid and of calcium, which is of no diagnostic significance, however. Variations in the uric acid content of the urine are insignificant.

ASTHMA BRONCHIALE

Essential Factors.—Blood eosinophilia; presence of Curschmann spirals and Charcot-Leyden crystals in the sputum; sputum eosinophilia.

The Blood.—*The Red Cells and Hemoglobin.*—In cases of asthma with cyanosis there may be a marked polycythemia with correspondingly high hemoglobin figures; otherwise, normal values are seen, or in some instances a moderate degree of secondary anemia. The highest figures are obtained during the paroxysms.

The Leukocytes.—The number of the leukocytes between attacks is normal, while during the paroxysms and immediately thereafter they are usually increased to from 10,000 to 20,000. Occasionally higher values are met with. Coler cites a case in which 52,000 were counted, but such an occurrence is exceptional. The differential count shows the existence of a distinct *eosinophilia* which seems to appear shortly before the paroxysms and persists for a variable time thereafter. In most cases it disappears between attacks, but in some instances higher counts than normal have also been noted in the intervals. 10 to 20 per cent. may be regarded as average values, but in some cases much higher figures have been recorded. In a case reported by Billings 53.6 per cent. were noted with a total count of 8300. Such high values, however, are unquestionably rare, and would ordinarily excite suspicion of the existence of an associated parasitic disease.

The eosinophilia of bronchial asthma is an important factor in the diagnosis of the disease, as it is absent in similar types of dyspnea referable to cardiac and renal disease. In emphysema, on the other hand, the same condition occurs.

Between attacks the mononuclear elements are usually increased.

The Sputum.—In many cases of asthma there is no sputum whatever during the paroxysms. When present it is usually scanty in amount, occurring in the form of glairy, grayish, mucoid masses, the perles de Laennec, which may be seen floating about in the spit-cup. As the attack breaks, sputum appears even in those cases where it was absent during the paroxysm. It is then fairly abundant, as much as 200 c.c. in the twenty-four hours, quite clear, thin, and frothy, and contains mucopurulent masses in variable amount. Within the next few days the secretion diminishes and then usually disappears, although occasionally the expectoration may be more or less continuous.

On careful examination the sputum will be found to contain Curschmann spirals, mucous moulds of the smaller tubes, and occasionally fibrinous casts of the bronchioles. These structures are most commonly found in the expectoration which first appears with the break of the attack, but may in some instances occur at any time thereafter. Some of the pearls above referred to will be found to be spirals. At one time the spirals were thought to be pathognomonic of bronchial asthma, but they are now known to occur also in acute and chronic bronchitis, in croupous pneumonia, and in chronic phthisis, though to a far less extent, so that their presence in fair number may still be regarded as quite suggestive.

Of some diagnostic import also is the presence in the sputum of large numbers of eosinophilic leukocytes. In typical cases of bronchial asthma these are, indeed, the predominating cells in the sputum. In fresh material they are usually well preserved, but even then it will be seen that eosinophilic granules are scattered over the entire microscopic field. Very curiously, many, if not most, of the cells are mononuclear; they are not myelocytes, however, but mononuclear histogenetic forms. They may be found at practically any period of the paroxysm. Their presence in predominating numbers, as I have just said, is of some diagnostic significance, but it should be remembered that they may occur in fairly large numbers also in other pathological conditions. Teichmüller speaks of their presence in tuberculosis (which see) and has further described an "eosinophilic" bronchitis, in which the sputum is said to differ markedly from that of bronchial asthma. (See Bronchitis.) Further studies in this direction are indicated.

Mast cells have also been noted in the sputum of bronchial asthma, but do not seem to be numerous. Alveolar epithelial cells, often showing marked myelin degeneration, are fairly numerous when the sputum has become more abundant. Hemorrhage of slight extent is noted in about 25 per cent. of all cases.

It is generally stated in the text-books that Charcot-Leyden crystals may be found in those asthmatic sputa which contain eosinophilic leukocytes in large numbers. My experience has not borne this out; many cases occur in which the eosinophiles are very numerous, in which no crystals can be found in the fresh material. On standing, however, they commonly develop. Their presence, like that of the spirals, was once regarded as pathognomonic of asthma; they were, indeed, supposed to stand in a causative relation to the disease. This view has now been abandoned, and it is known that they may be found in other diseases as well; nevertheless, their presence is much more common in asthma, and they accordingly deserve some diagnostic consideration. Crystals of oxalate of lime and of calcium phosphate are also occasionally seen.

The Urine.—In advanced cases in which emphysema has developed and the heart has become insufficient, albuminuria and cylindruria may be observed. Edelmann reports that in 4 cases which were studied a slight turbidity of the urine was noticed following the attack of asthma. Microscopic examinations showed this to be referable to the presence of leukocytes, many of which were eosinophiles. In 1 case the blood contained only 8.5 per cent., while in the urine they numbered 30 per cent.

BARLOW'S DISEASE (INFANTILE SCURVY)

Essential Factors.—Chlorotic anemia; hyperleukocytosis; lymphocytosis.

The Blood.—*The Red Cells and Hemoglobin.*—In Barlow's disease, as in ordinary scurvy (which see), there may be intense *anemia;* in the milder cases, however, the red count is occasionally normal. In seven cases studied by Da Costa the figures ranged between 2,950,000 and 5,100,000 (3,527,000 average) and the hemoglobin between 35 and 65 (average 43) per cent., thus giving an average index of 0.57. In a case described by Reinert the red cells fell to 976,000 and the hemoglobin to 17 per cent.

The Leukocytes.—The leukocytes in Da Costa's cases varied between 8000 and 25,000 (average 15,557) all but one showing a decided increase. In 4 of the 7 the percentage of lymphocytes was between 60 and 66; in the three that of the neutrophiles lay between 27 and 35; the eosinophiles were minimal normal, and in all but one myelocytes were present, ranging from 1 to 6 per cent. (average 2.5).

The Plaques.—The plaques are not diminished and *coagulation* is normal.

The Urine.—Shows no special abnormalities.

BASEDOW'S DISEASE

Essential Factors.—Lymphocytosis; presence of protective ferments in the serum, reacting with Basedow's thyroid and usually with thymus.

The Blood.—A moderate grade of chlorotic anemia—thyroid chlorosis (Capitan)—is observed in some cases, but is not a constant feature of the disease. In Cabot's series of eighteen cases the lowest red count was 3,483,000, with 50 per cent. of hemoglobin; the average value was 69 per cent. Zappert records two cases with a red count lower than 3,000,000 and 30 to 32 per cent. of hemoglobin. Ewing reports that he once found the red cells undersized in such an anemic instance of the disease.

The total leukocyte count is usually not increased beyond 10,000; in most cases the values are normal, and not infrequently they are subnormal. Occasionally a definite hyperleukocytosis is observed, for which an explanation is not apparent. Cabot thus mentions a case with a count of 23,100.

The differential count frequently shows a marked lymphocytosis which may be absolute as well as relative. Kocher lays some stress upon this factor in the prognosis of the disease.

Occasionally a moderate increase of the eosinophiles has been observed, but I am inclined to regard this as accidental.

Serology.—Lampé and Papazolu have shown that the blood serum of Basedow patients contains ferments which react with Basedow thyroid and almost always also with thymus, and in female patients usually with ovary. The degree of reaction is variable. Sometimes the maximal reaction is obtained with thyroid, sometimes with

thymus, and occasionally with ovary. In 1 case in which there was marked muscular atrophy Lampé and Fricks obtained a very marked reaction also with muscle antigen. Generally speaking, the most pronounced reactions are found in severe cases. Several Basedowoid cases showed no reaction whatever.

The Urine.—In some of the cases there is marked polyuria, but in the majority the amount is normal. The urea, uric acid, and ammonia content remain unchanged. Albuminuria is exceptional. According to several observers there is a certain tendency to glucosuria of the digestive type and in some instances true diabetes has been observed to develop. Acetone has occasionally been observed in small amount

The Feces.—In several cases of Basedow fatty stools have been observed (Falta, 6 cases; Bittorf 1 case). In Bittorf's case there was almost a total absence of trypsin and an excess of muscle fibres in the feces, showing that external pancreatic secretion was seriously at fault.

BERI-BERI (KAKKE)

The Blood.—Detailed studies of the blood of beri-beri patients are still wanting. Spencer states that in the majority of cases there is a well-defined anemia with at times marked changes in the size and form of the red corpuscles. The leukocytes are not increased and the differential formula is normal, excepting during the acute stage of the disease when eosinophilia may be observed. Takasu mentions the occurrence of basophilic granular degeneration, but to judge from the abstract of his article in the *Folia Hematologica* (Vol. I), this was relatively slight, scarcely exceeding what one may at times observe in supposedly normal individuals. He mentions, however, that stiple cells do not occur in chronic cases. The leukocytic picture in adult cases, according to the same writer, is normal.

Regarding his findings in sucklings the following data are of interest: 29 positive cases and 6 doubtful cases were studied. In 11 the hemoglobin varied between 75 and 100 per cent.; in most cases it was higher than 90. In 17 the red cells varied between 2,440,000 and 4,800,000; in two-thirds the count was above 3,500,000. The leukocytes ranged between 9000 and 34,000, the variations in two-thirds of the cases being between 11,000 and 17,000. The polynuclears were diminished and the lymphocytes increased to more than twice the number of the former. The eosinophiles were usually low—in half of the cases less than 1 per cent.; only in six cases was there an increase (6 to 8 per cent.).

The Urine.—Even in the dropsical cases the urine contains no albumin or only mere traces.

BILHARZIASIS

Essential Factors.—Hypereosinophilia; large mononucleosis; hematuria; presence of bilharzia eggs in the urine.

The Blood.—In advanced cases of bilharziasis the patients become markedly anemic, but in early cases or such of mild severity there may be no anemia.

The Leukocytes.—The leukocytes may or may not be increased. In one case mentioned by Manson the count was 8200. Douglas and Hardy speak of leukocytosis. The eosinophiles are increased in almost all cases. In 22 cases, uncomplicated by uncinariasis, Kautsky-Bey found 5 per cent. as minimum and 53 as maximum. In the majority of cases the values were between 10 and 20. This is in accord with the results of Douglas and Hardy, who found an average of 16.5 and values lower than 6 per cent. in only two out of 50.

The large mononuclears are increased, even though malaria does not complicate the case. Douglas and Hardy found 12.5 per cent. as average.

The Urine.—The most characteristic symptom of bilharziasis is the passage of blood at the end of micturition, with or without a sense of urinary irritation. The amount so passed varies from a few drops of slightly tinged urine to a considerable quantity of pure blood. As a rule, only the last few drops of urine contain blood; sometimes, however, the hemorrhage is more considerable, when the entire bulk of the urine may be tinged. Occasionally clots even are passed (Manson). On microscopic examination the spined ova of the Bilharzia hæmatobia (Fig. 55) are found in the urinary sediment, which may conveniently be obtained by centrifugation. Sometimes only a few are seen, while at others they are very numerous. In severe cases the condition is usually complicated, sooner or later, by cystitis, prostatitis, seminal vesiculitis, and pyelitis, with corresponding urinary findings.

The Feces.—When the rectum is involved dysenteric-like symptoms may supervene, mucus with blood being passed from time to time, the stools becoming frequent and their passage attended with tenesmus. In such cases small, soft growths are to be felt inside the sphincter ani. On removing one of these and breaking it up with needles, the spined ova can be made out in the debris (Manson).

Vaginal Discharge.—In the female the ova may be found in the vaginal discharge.

BRAIN TUMORS

Essential Factors.—Irregular anemia; absence of hyperleukocytosis; normal cytological findings in the cerebrospinal fluid of non-syphilitic cases; lymphocytosis; positive Wassermann and butyric acid reaction in syphilitic cases.

The Blood.—The blood picture in brain tumor depends to a considerable extent upon the nature of the individual case. When the tumor is secondary to disease elsewhere in the body the character of the primary growth will largely determine the findings, which accordingly are more or less variable. This is true to a certain extent even of those cases in which the brain lesion is primary. The available data in the different types, moreover, are as yet too meager to speak definitely upon the subject. It appears, however, that in most cases a certain degree of anemia is quite usual. In 5 cases observed by Da Costa the hemoglobin ranged from 70 to 79 per cent. (average 72.2) and the red cells from 2,860,000 to 4,270,000 (average 3,800,000), while the leukocytes were not increased in any. Cabot found a hyperleukocytosis varying from 10,400 to 18,100 in 15 cases out of 24; in 7 of these the count was 15,000 or higher. Of the differential findings practically nothing is known.

In the syphilitic cases a positive Wassermann reaction may be expected without exception.

Cerebrospinal Fluid.—In cerebral tumors which are so located as not to interfere with its circulation the amount of the fluid is usually quite large; it is perfectly clear and usually of low specific gravity. The amount of albumin is small, varying from mere traces to 0.8 pro mille. The cytological formula shows no deviation from the normal, excepting in syphilitic cases, where lymphocytosis is the rule. The Wassermann reaction applied to the cerebrospinal fluid is positive only in the latter cases. In these Noguchi's butyric acid test and the Ross Jones test will also be found to be positive.

The Urine.—In some of the cases, slight albuminuria may be observed and transitory glucosuria may occur in connection with tumors about the base of the brain.

BLASTOMYCOSIS

The Blood.—The blood picture depends upon the stage of the disease and its extent. As the malady progresses a secondary anemia develops which may become quite severe. Stober thus mentions a case in which in consequence of repeated hemorrhages the hemoglobin fell to 12 per cent.

The Leukocytes.—The leukocytic picture likewise varies with the stage of the disease, its extent, the existence of complications, etc. Stober gives 16,800 as average, excluding cases in which sepsis distorted the picture. In those cases in which the total count is little or not increased the mononuclears are apt to be somewhat increased. Stober's average figures were 17 per cent. for the small monos, 7 for the large monos, 73 for the polynuclear neutrophiles, 1.5 for the eosinophiles and 0.1 for the basophiles.

The Pus.—In the pus of the corresponding ulcers and abscesses the organism causing the disease is found, occurring in pairs or clumps of individual round or oval bodies, as described in the section on the Sputum.

The Sputum.—The findings in blastomycosis of the lung are essentially the same as in pus. The sputum is usually thick and purulent or mucopurulent in character and frequently contains blood in small amounts.

BRONCHIECTASIS

Essential Factors.—Irregular anemia and hyperleukocytosis; abundant expectoration with tri-sedimentation; presence of fatty acid crystals, and occasionally of cholesterin, leucin, and tyrosin; usually absence of elastic tissue; absence of tubercle bacilli.

The Blood.—The blood picture in bronchiectasis depends upon the nature of the underlying malady, the frequency and extent to which hemorrhages have taken place, and the duration of the condition. Often there is marked anemia, which may in part be obscured by a relative polycythemia, referable to associated cyanosis. The leukocytes are no doubt frequently increased, although I have not been able to find actual counts in the literature. The septic factor also must unquestionably be common.

The Sputum.—This is usually very abundant and expectorated in "mouthfuls" at a time. Commonly it is brought up in paroxysms, during which the cavities are more or less emptied; this at leas is the case in the saccular cases to which our account has reference more particularly. In the cylindrical cases the findings are less characteristic.

The daily amount varies considerably and seems to depend neither upon the duration of the disease, nor upon the size or number of the cavities. In the Hopkins series of twenty-three cases, mentioned by Emerson, it was less than 100 c.c. in one (15 to 30 c.c.), in eleven it varied between 100 and 300; in two it was almost 500 and in seven over 600 c.c. In one of the cases it frequently exceeded 1000 c.c. Toward the fatal end the amount may rapidly diminish. On standing, the expectorated material shows typically a sedimentation into three layers, the lowest of which is purulent, the middle layer serous, and the surface layer frothy and mucopurulent; streamers of mucopurulent material frequently extend downward into the second layer. This tri-sedimentation is the rule when the disease has become well established, while in the earlier stages it may be imperfect, and before infection of the cavity takes place the sputum may be purely mucoid. The color of the sputum, as a whole, is a grayish-greenish yellow, with here and there areas of red or brown, which are referable to blood in more or less altered condition. Usually the bleeding is slight, but at times extensive hemorrhages take place. Emerson

mentions a man who was admitted to the hospital fourteen times, and five times owing to hemorrhages which threatened his life. In another instance 1700 c.c. were lost in about ten minutes. The odor is commonly insipid and sweetish, but at times the material emits a horrible stench, when extensive putrefactive changes have taken place in the cavities.

Microscopic examination in a well-developed case, after infection of the cavity has occurred, shows the presence of pus cells in enormous numbers, many of them well preserved, but many others in various stages of degeneration. Alveolar epithelial cells may be numerous in early cases, but later the mucosa is destroyed. Red cells are almost always present in variable number. Bacteria are found in immense numbers, frequently massed in extensive zoöglea. Fatty acid crystals are common and often large; they are especially abundant in little cheesy particles, the so-called plugs of Dittrich. Hematoidin, leucin, tyrosin, and cholesterin may likewise be encountered. Elastic tissue is only exceptionally present. Occasionally, calcareous concretions are formed in the cavities and may be brought up with the sputum. Curious examples of this kind have been reported. Andral cites a case of phthisis in which within eight months 200 stones were expectorated, and Portal mentions a case where 500 were thus expelled. Their size varies; in one of the Hopkins cases they were about the size of a split pea.

The Urine.—The urine shows no essential changes which could be referred to the existence of bronchiectatic cavities *per se*, but it stands to reason that in the long run the patient's general health must suffer more or less, so that secondary changes may result in various organs. Albuminuria and cylindruria are then not uncommon. In some cases, where putrefactive changes are especially extensive, and absorption fairly active, the elimination of fatty acids may be much increased; the same would probably hold good for the conjugate sulphates, although I have not seen any data bearing on this point.

BRONCHITIS (ACUTE)

Essential Factors.—Irregular leukocytosis and differential findings, depending upon the nature of the infecting organism; mucoid or mucopurulent expectoration containing the offending microörganisms.

The Blood.—*The Red Corpuscles and Hemoglobin.*—An ordinary attack of acute bronchitis rarely gives rise to notable anemia, but a loss of hemoglobin amounting to a few points is probably a common event.

The Leukocytes.—The behavior of the leukocytes depends upon the nature of the offending microörganism and the intensity of the infection. The pneumococcus and catarrhal micrococcus produce a hyperleukocytosis which, in the case of the former especially, may

be just as extensive as in pneumonia (20,000 to 40,000); the differential count shows the septic factor, viz., an increase of the neutrophiles associated with a decrease or absence of the eosinophiles. In infections with the influenza bacillus, on the other hand, there is rarely a hyperleukocytosis of any moment; usually the count is maximal normal, while the leukocytic formula shows a marked lymphocytosis (40 to 60 per cent.).

The Sputum.—Early in the attack the sputum is colorless, transparent, scanty, mucoid, and highly tenacious, so that it is often possible to invert the cup without spilling the contents—the *sputum crudum* of the ancients; occasionally it is streaked with blood. Microscopic examination shows the presence of a few leukocytes, red corpuscles, and a variable number of ciliated epithelial cells, in some of which myelin droplets can be distinguished. Occasionally the cilia may be seen in motion. In some cases the character of the sputum remains mucoid throughout the attack, if, indeed, there is any at all; but, as a general rule, it increases in amount and becomes progressively more purulent as the bronchitis continues. At the height of the disease the quantity is usually between 100 and 200 c.c. The color, owing to the presence of the pus, becomes yellow or greenish yellow, and for the same reason its transparency disappears. Microscopic examination at this time reveals the presence of large numbers of polynuclear neutrophiles, with here and there an eosinophile. The epithelial elements, in so far as they are derived from the bronchi, have lost their characteristic appearance; they are now roundish and frequently filled with fat globules or myelin; this component may also be present in considerable amount in the free state. A variable number of red cells and alveolar epithelial cells are frequently found.

With the development of convalescence the sputum becomes progressively looser, viz., less mucoid, more purulent, and gradually diminishes, until normal conditions are restored. Certain deviations from this picture may, of course, occur, but are, on the whole, unimportant. Teichmüller has described an "eosinophilic bronchitis" which, as the name implies, is characterized by the presence of large numbers of eosinophiles. I have seen a few cases of this kind, and found, as in bronchial asthma, that the majority of the cells are mononuclear and manifestly of histogenetic origin. Their significance is not clear. Typical spirals are absent, but rudimentary forms may be encountered. Teichmüller states that Charcot-Leyden crystals may be present. The organisms which most commonly predominate in the sputum are the catarrhal micrococcus, the pneumococcus, streptococci, and the influenza bacillus.

The Urine.—The urine presents no special abnormalities, being essentially of the febrile character. Albumin is only exceptionally found, unless some other underlying disease exists which in itself gives rise to albuminuria.

BRONCHITIS (CAPILLARY)

Essential Factors.—Irregular leukocytosis and differential findings; mucopurulent expectoration.

The Blood.—*The Red Corpuscles and Hemoglobin.*—Capillary bronchitis developing secondarily to other pathological conditions, such as measles, whooping cough, diphtheria, etc., is very commonly associated with a certain grade of anemia. It is especially noticeable in those cases which develop in tubercular and rickety children. A primary capillary bronchitis, on the other hand, occurring in an otherwise healthy subject, does not lead to any marked loss of red cells and hemoglobin.

The Leukocytes.—These are usually much increased, the number varying between 15,000 and 41,000. The differential formula depends upon the nature of the offending organism, and the character of the underlying disease. Many cases present the septic factor, but in a very considerable number there is a marked lymphocytosis. In the former event it may be impossible to distinguish the condition from ordinary pneumonia.

The Sputum.—In young children the sputum is swallowed. In older individuals there is a small amount of mucoid or mucopurulent expectoration, which is often brought up with much difficulty.

The Urine.—The urine presents no special peculiarities which could be attributed to the bronchitis *per se.*

BRONCHITIS (CHRONIC)

Essential Factors.—Irregular secondary anemia and leukocytosis; mucopurulent sputum; bronchorrhea.

The Blood.—*The Red Corpuscles and Hemoglobin.*—Chronic bronchitis *per se* is not very apt to produce anemia, but as the condition is frequently secondary to some other disease which in itself may cause anemia, the blood count and hemoglobin values are sometimes found diminished.

The Leukocytes.—The number of the leukocytes is similarly influenced. Various writers state that hyperleukocytosis is uncommon in chronic bronchitis, but in Cabot's series of twenty-six cases counts exceeding 10,000 were noted in 17, and in 5 of these they were higher than 18,000, reaching 38,000 in one. The writer, nevertheless, remarks that he thinks if more counts had been added nearly all would have been normal. The whole question undoubtedly hinges upon the nature of the offending microörganism, which also determines the leukocytic formula. Where asthma and emphysema are underlying conditions eosinophilia may be observed. In other cases a lymphocytosis is noted, and in still others the septic factor.

The Sputum.—Early in the disease the sputum is mucoid, very tenacious, colorless, and transparent, as in the acute cases, and it may

remain so for a long time. This is true especially of the subacute cases. When the disease has become chronic, however, the sputum tends to be mucopurulent and of a yellowish-greenish color. During periods of improvement there is a return to the mucoid condition, while exacerbations of the disease render it more and more purulent. The amount is variable. Sometimes there is only a little expectoration early in the morning, while at others the quantity is large; 100 to 200 c.c. per day is common. Occasionally one can speak of a true *bronchorrhea,* when 300 to 500 c.c. or more is expectorated in the twenty-four hours. In these cases there is very little mucoid material, the sputum consisting almost of pure pus. On standing it is apt to separate into three layers—the purulent material at the bottom, with a dirty serous layer above and a more or less frothy and muco-watery layer on top. Ordinarily the sputum of chronic bronchitis has little or no odor, but at times it is quite disagreeable; still there is never that stench which is observed in cases of putrid bronchitis with bronchiectasis, in gangrene and abscess or in those cases where an empyema has perforated into the lung.

Microscopic examination shows the presence of large numbers of pus cells—polynuclear neutrophiles—in all stages of degeneration, with here and there an eosinophile, some red blood cells, and epithelial cells filled with fat or myelin globules, which latter, moreover, are present in variable quantity in the free state.

Bacteria are always present in enormous numbers. The majority are probably harmless saprophytes, but among them there are pathogenic organisms which are in part responsible for the pathological condition; pneumococci are frequently abundant; in other cases one meets with streptococci, catarrhal micrococci, staphylococci, influenza bacilli, etc.

The Urine.—The urine shows no abnormalities which are referable to the chronic bronchitis *per se.*

BRONCHITIS (FETID)

The laboratory findings in fetid bronchitis are essentially the same as in bronchiectasis. Here as there the amount of sputum is usually large, there is distinct tri-sedimentation, and on microscopic examination one finds enormous numbers of pus corpuscles, innumerable bacteria (among them often long threads of leptothrix), and fatty acid needles; the latter are especially abundant in the so-called plugs of Dittrich which may be found in the lowest layer of the sputum. The odor is fetid and very penetrating. Elastic tissue is absent, unless gangrene of the lung complicates the case (which see). As the opportunities for resorption are rather better in fetid bronchitis than in bronchiectasis the patients become anemic at an earlier date, and I doubt not that hyperleukocytosis is a constant factor during the active periods of the disease.

BRONCHITIS (FIBRINOUS, ACUTE)

Essential Factors.—Presence of fibrinous casts in the sputum.

The Blood.—The blood findings in acute fibrinous bronchitis depend upon the underlying condition, such as typhoid fever, erysipelas, measles, smallpox, scarlet fever, acute articular rheumatism, exophthalmic goitre, pulmonary tuberculosis, mitral disease, and need not be considered at this place.

The Sputum.—The disease receives its name from the occurrence in the sputum of casts of the bronchi, which supposedly consist of fibrin; attached to these there are often numerous epithelial cells and variable numbers of leukocytes and red cells. In pneumonia these formations are frequently observed immediately before or after resolution has taken place, and in diphtheria they are seen when the membrane has extended into the finer ramifications of the bronchi. Occasionally they are found following the inhalation of irritating vapors, and at times also in those rare cases of albuminous expectoration which follow thoracentesis. (See general chapter on Sputum.)

BRONCHITIS (FIBRINOUS, CHRONIC)

Essential Factors.—Presence of fibrinous casts in the sputum.

The Blood.—The blood shows no abnormalities.

The Sputum.—The sputum in these cases is essentially that of a subacute bronchitis, to which is added the occasional expectoration of bronchial casts. These sometimes only appear at intervals of several months, while at others one or more may be expectorated on one day. The amount of sputum which is brought up on such occasions is sometimes quite considerable, even exceedinging a pint in the twenty-four hours. It is muco-watery and commonly contains some blood, which is brought up with the cast. Curschmann spirals may be simultaneously present and continuous with branches of the cast. Microscopic examination shows the presence of epithelial cells which may be attached to the cast in large numbers; this, however, is not necessarily the case. In addition, there are leukocytes, in some cases many of them eosinophiles, red blood cells, hematoidin crystals and granules, particles of lecithin, and not infrequently Charcot-Leyden crystals.

BRONCHOPNEUMONIA

The laboratory findings in bronchopneumonia are essentially the same as those noted in capillary bronchitis. At times the leukocytosis is most extensive. In one case, complicating whooping cough (reported by Cabot), the count rose to 94,600, with 66 per cent. of lymphocytes. In another, a baby, aged fifteen months, the initial

count was 103,000, and rose to 185,000 on the fifth day; here also there was a marked lymphocytosis (64.5 per cent.). The first child recovered, while the second died. Sometimes the sputum resembles the rusty material seen in lobar pneumonia, but this is exceptional; more commonly it is mucoid or mucopurulent, and at times, notably in young children, there is none. In the tubercular cases bleeding from the lungs occurs early, and it may be possible to demonstrate tubercle bacilli and elastic tissue before the disease has advanced very far.

In bronchopneumonia the organisms which are most frequently encountered are the Staphylococcus aureus, the catarrhal micrococcus, streptococci, and the bacillus of Friedländer.

BUBONIC PLAGUE

Essential Factors.—Irregular polycythemia; hyperleukocytosis with septic factor; delayed coagulation; presence of plague bacilli in the blood, glands, sputum, and urine; agglutination reaction after the first week.

The Blood.—*The Red Cells and Hemoglobin.*—Unless septic complications supervene there is no decrease in the number of the red cells; usually the count is normal or there may be a relative polycythemia of variable extent. Aoyama reports counts varying from 4,400,000 to 8,100,000. The hemoglobin is usually somewhat reduced; according to the findings of the Austrian Commission, to from 65 to 80 per cent.

The Leukocytes.—The leukocytes are increased in all but the most malignant and the most benign cases. In the latter the count may be normal, and in the intensely toxic cases there may be actual leukopenia. The degree of increase in cases of average severity is variable. The Austrian Commission gives 12,000 to 28,000 as usual values; Rogers, 20,000 to 60,000; while Aoyama cites 4 cases in which the number exceeded 100,000, reaching 200,000 in one instance. His average (6 cases) accordingly is much higher than that of the other observers, viz., 96,000. As regards the differential findings, there seems to be some difference of opinion. Aoyama states that the increase is of the neutrophilic type with marked diminution of the eosinophiles. Ewing found the same, while Rogers speaks of a lymphocytosis, and remarks that in most cases the neutrophiles showed little or no increase. Another observer, Zinno, reports the occasional occurrence of an eosinophilia. This can hardly be surprising in countries where infections with intestinal parasites are so common, and it stands to reason that a superadded bacterial infection may not always bring the eosinophiles below normal, nor even to normal.

The Plaques.—The plaques are usually much increased.

Coagulation.—Coagulation is said to be very slow. According to Corthorn it failed entirely in 10 of 12 fatal cases.

Bacteriologic Examination.—In advanced cases of bubonic septicemia the specific organism may be found in the blood in small numbers. Toward the end of rapidly fatal cases they become more numerous, and may then be demonstrated directly with the microscope. According to Bell the bacilli can be found in all cases and at all stages of the disease by using Ross' dehemoglobinizing method (which see). The Austrian Commission, on the other hand, regards this direct examination as liable to lead to error, and has pointed out that in many cases where this gives a positive result cultural methods with blood from a vein may show the opposite, which suggests that the organism in question may be referable to contamination. Further studies in this direction are accordingly necessary.

Agglutination Test.—Labolotny has shown that the serum of bubonic patients will agglutinate the corresponding organisms in a large number of cases. From the standpoint of early diagnosis the reaction is not of much interest, however, as it is absent during the first week. In the second week he obtained positive results with a dilution of 1 to 10, while in the third and fourth week the organisms were clumped with a dilution of 1 to 50.

The Sputum.—In cases of the pneumonic type the plague bacillus is seen in the sputum in enormous numbers and may be found already on the first day of the disease. By direct examination, however, it may not be recognized immediately, and it is best in every case to resort to culture as well. (See Plague Bacillus.)

The Pus.—For diagnostic purposes it is well in all cases, excepting the pneumonic, where the organisms can be readily obtained from the sputum, to attempt its isolation by aspirating one of the glands, if suppuration has already begun, or to make a culture directly from a gland that has been exposed by a small incision.

The Urine.—The urine is scanty, but rarely contains more than traces of albumin (Manson). In some cases the corresponding organism has been found.

BURNS

Essential Factors.—Polycythemia; marked hyperleukocytosis with normal differential counts soon after the accident, and with the septic factor later on.

The Blood.—In cases of severe burns there is usually a fairly well-pronounced *polycythemia,* due, no doubt, to vasomotor disturbances and altered blood distribution. In Locke's series of 10 cases the counts ranged between 4,500,000 and 9,250,000; in 8 the number was higher than 6,000,000. It is noteworthy that this effect may be obtained already one hour and a quarter following the accident.

The Leukocytes.—The leukocytes are likewise increased in practically every case. This increase is also in part at least due to vasomotor influences, as is evident from the fact that the corresponding differential counts may show normal values; this is noticeable especially soon after the accident. In one instance (No. 6 of Locke's series) the count was 30,000 thirty minutes after the accident, while the differential count showed 23.3 per cent. of small mononuclears, 7 per cent. of large mononuclears, including the so-called transition forms, 67 per cent. of neutrophiles, and 2.6 per cent. of eosinophiles. An influx of leukocytes from the bone marrow usually follows soon after, however, as is shown by the increased percentage of neutrophiles; the eosinophiles then diminish and are apt to disappear, so that a typically septic blood picture develops. The total values in Locke's series varied between 7000 and 78,000; in all but one they were above 15,000. Myelocytes in small numbers may be met with in isolated cases. As the case progresses these blood changes are apt to persist to a greater or less extent, and are in proportion to the degree of subsequent suppuration.

The Plaques.—The blood plates are markedly increased.

Hemoglobinemia.—In some instances hemoglobinemia has been observed.

The Urine.—In extensive burns the urine may contain a considerable amount of albumin and a large number of casts of all kinds. In some instances hemoglobinuria has been observed. Wilms speaks of albumosuria as the usual event after severe burns, and as appearing immediately after the injury. The toxicity of the urine is said to be increased.

CANCER

While the various laboratory findings in cases of cancer are essentially dependent upon the seat of the disease, and will be discussed in some detail under the corresponding headings, there are certain general features, referable more directly to the cancerous process *per se*, which may be appropriately discussed at this place.

Essential Factors.—Secondary chlorotic anemia; occurrence of erythroblasts; increased sugar content of the serum; presence of specific meiostagmins; increased content of antitrypsin; presence of isohemolysins associated with increased antihemolytic resistance of the autologous corpuscles; complement fixation; presence of protective ferments in the serum; absence of hydrochloric acid from the gastric juice; increased nitrogenous katabolism.

The Blood.—*The Red Cells and Hemoglobin.*—While anemia develops sooner or later in every case of cancer, it is important to bear in mind that this is not necessarily an early symptom. Its appearance in many cases is manifestly not referable so much to the existence of the disease *per se* as to its interference with the normal

activity of certain organs, and more particularly the organs of digestion. It is thus noteworthy that in many cases of cancer of the breast the patient's color is excellent, even though the regional lymph glands be already involved, and that a material anemia does not develop until metastasis to the internal organs has manifestly occurred. On the other hand, it is sometimes most striking to note the extensive anemia which may be observed in relatively early cases of cancer of the stomach, where neither the extent of the primary lesion, nor corresponding metastases seem sufficient to explain such extreme destruction of the blood corpuscles. In another group of cases it is impossible to decide what share, if any, the cancerous process has had in the production of a severe anemia, since the occurrence of hemorrhages and superadded infections in themselves are quite sufficient to account for its existence. In advanced cases the appearance of the patient in itself furnishes a better insight into the extent of the anemia than does the blood count, since, owing to the extreme desiccation of the individual there is a concentration of the blood and hence a relative polycythemia. The most extensive grade of polycythemia of this order is seen in esophageal cancer, where the number may rise to 7,000,000 or even higher. The lowest values occur in cancer of the stomach, and in association with septic complications in cancer of the uterus. Counts between 1,000,000 and 2,000,000 are here not uncommon. In cancer of the stomach, however, the count does not often drop below 1,500,000. Henry has drawn attention to this fact, and has insisted upon its importance in the differential diagnosis of this condition from pernicious anemia, where the red cells in fatal attacks fall below 1,000,000. In a general way this holds good, but there are exceptions on both sides. Average figures regarding the number of red cells in cancer in general are hence of little value.

The *hemoglobin* values are almost always lower than the corresponding red counts, and it is noteworthy that the oligochromemia appears earlier than the oligocythemia. In extreme cases the loss of coloring matter is most extensive, falling to 20 per cent. and even lower. The color index accordingly is diminished. In Da Costa's series of 145 cases the average was 0.86, and according to the same writer it is generally lower in the early than the late stages of the disease. Values above 1 are exceptional.

Morphological examination shows no essential changes excepting in markedly anemic cases. In these the anemia of the individual corpuscle is usually quite apparent and corresponds to the lowered color index. True poikilocytosis may occur, but it is rarely so extensive as in pernicious anemia; an increased susceptibility to mechanical insults, on the other hand, is not infrequently recognizable. Anisocytosis may be marked, but the deviation in size is in the direction of undersize rather than toward oversize. Stiple cells

are scarce, while polychromatophilic cells are more common and especially so in the septic cases.

Nucleated red cells, while usually not numerous, are found in a large percentage of the cases and frequently at a time already when the anemia is not extensive. This factor serves to distinguish cancer anemia from the majority of the other forms of secondary anemia; it is, however, not constant. The cells are usually normoblasts, but in some cases a few megaloblasts may be seen; if so, they are less numerous than the former.

The Leukocytes.—The leukocytes show no constant changes in cancer. In some cases they are increased, in others normal. Some writers believe to have demonstrated that the seat of the disease is the *primum mobile* in this direction, while the results of others do not warrant this inference. Where septic complications exist, or where hemorrhages have taken place, a hyperleukocytosis would, of course, be expected, but the high values which are found in some cases cannot always be accounted for on this basis. Anemia *per se* is not an essential causative factor. My own impression has been that the occurrence or non-occurrence of hyperleukocytosis will depend to a great extent upon the amount of necrotic material which is in existence and upon the degree of resorption. In skin cancers where this factor plays the smallest role we accordingly find that there is no increase, while cancer of the internal organs, notably the larger glandular organs, is more apt to be associated with increased values. (For a consideration of the digestive leukocytosis in cancer, see Cancer of the Stomach.)

In those cases in which hyperleukocytosis occurs the increase is referable to the neutrophilic elements. The eosinophiles in some of these cases are diminished or disappear entirely, while in others they persist. To the latter occurrence I am inclined to attach some diagnostic significance in suspected cases. In cases with normal leukocyte counts the differential count may be normal; in others I have noted maximal lymphocyte values, and in several cachectic cases I found the large mononuclears high (15 to 20 per cent.). A few neutrophilic myelocytes are frequently seen in anemic cases, but are of no special significance; large numbers may be met with when bone involvement exists.

The Plaques.—The plaques are inconstant in their behavior; sometimes they are normal, sometimes diminished, sometimes increased; they may vary in the same case.

Coagulability.—The coagulability of the blood is not altered in uncomplicated cases, nor is the amount of *fibrin*, while the *specific gravity* runs a course which is roughly parallel to the degree of anemia. The *alkalinity* is said to be decreased in cancer of the stomach, but this has not been proved.

Chemical Examination.—According to Trinkler, this shows the presence of large quantities of a reducing substance, the greater portion of which consisted of *glucose* (average 0.1819 per cent., 0.303 maximum.) This was more abundant in cancer of the internal organs than in cancerous disease of the skin and the mucous membranes, and did not seem to bear any relation to the degree of cachexia. The results of the same writer apparently also bear out the conclusions reached by Freund, who claimed that a differential diagnosis between carcinoma and sarcoma can be made upon this basis, since sarcoma does not give rise to an increased sugar content of the blood.

Serology.—Within recent years several interesting deviations from the normal have been noted in the behavior of the blood serum of cancer patients, some of which merit a brief consideration.

Presence of Specific Meiostagmins.—According to Ascoli and Izar the blood serum of cancer patients contains specific meiostagmins, which can be detected by suitable methods. Positive results have been obtained in a large number of cases, and it seems as though the reaction was destined to play a role in diagnosis.

Micheli and Cattoretti have confirmed these results in 18 cases of cancer, the drop-plus varying from 1.2 to 5.4, as contrasted with a plus of 0.6 to 1.2 in the non-cancerous cases.

The Antitrypsin Content of the Serum.—The antitrypsin content of the serum is increased in a large majority of the cases. Brieger and Trebing found this in 91.6 per cent. and v. Bergmann and Meyer in 92.7. My own results have not been quite so favorable, viz., 77.3 per cent. Unfortunately a positive reaction may also be obtained in non-cancerous patients; according to my experience in 20 per cent. of all cases, selected at random. A positive reaction has thus a very limited value. The discovery of normal values, however, may very properly be regarded as strong presumptive evidence against the existence of cancer. As the technique is not at all difficult, I should suggest that the reaction be tried in all suspected cases.

The Presence of Isohemolysins in the Blood.—Through the researches of Weil, it has been established that the blood serum of cancer patients frequently contains hemolysins which are destructive for normal (*i. e.*, non-cancerous) corpuscles, while the patient's own corpuscles are more or less resistant. In a series of 31 cases, of which 15 were early and 16 late, the serum was found to be hemolytic in 46.5 per cent. of the former and 71.5 per cent. of the latter, while the patient's corpuscles were resistant in 80 per cent. of the late and 71 per cent. of the early cases. In non-cancerous diseases isohemolysins could be demonstrated in 21.5 per cent. of the cases, but in these the patient's own corpuscles were less strongly resistant than in cancer. Weil points out that if such easily identified conditions as pneumonia and advanced tuberculosis be excluded from this list

the (non-cancerous) figure falls to 12.5 per cent. Several investigators have repeated Weil's work and have come essentially to the same conclusions (Baumgarten, Janeway, Johnstone and Canning, Butler, Smithies, Schleiter), although it would seem that the list of diseases in which the reaction may at times occur is larger than was at first supposed. Crile alone seems to have obtained more favorable results, for he states that all early cases of malignant new-growth have a hemolytic serum, and that the corpuscles of such cases are immune to the destructive action of their own serum, or of the serum of other cancer cases. In advanced cases, according to the same writer, the reaction is apt to disappear. My own rather limited experience does not bear out Crile's assertions. Like the other observers mentioned, I found the reaction only in some 50 per cent. of the cancer cases. Of special interest is the discovery of Peskind that the blood serum of tertiary syphilitics frequently contains isohemolysins, and that the corpuscles of hemolytic luetic blood were found to be immune to the action of its own serum or any other syphilitic serum; and, furthermore, that in every instance the corpuscles belonging to a hemolytic carcinomatous blood were immune to the action of the hemolysins found in syphilitic serum; conversely the corpuscles of a hemolytic syphilitic blood were immune to the action of the hemolysins present in carcinomatous serum. In short, judging from the behavior of the sera toward the corpuscles derived from various normal and diseased persons, one could not distinguish a hemolytic syphilitic serum from a hemolytic carcinomatous serum.

From the available data it would thus seem that in the case of the hemolysins also a negative reaction is of more value in excluding malignant disease than a positive one is in affirming its existence, bearing in mind, however, that a positive reaction is only obtained in some 50 per cent. of the cases.

Heterolysins.—Kelling's work on the presence of heterolysins in cancer, viz., lysins directed against the corpuscles of other animals (chicken, sheep, ox) corresponds approximately to the findings in connection with the isohemolysins, just considered.

Complement Fixation.—While Thomas and myself had obtained a certain degree of complement fixation with the serum of cancer patients in about 57 per cent. of our cases, v. Dungern, with his more recent technique, claims much better and more specific results. In a recent publication he thus states that with the technique described in the first section of this volume he obtained a positive reaction in 91 of 101 cases (90 per cent.). Of these, 64 gave complement fixation already with $\frac{1}{20}$ c.c. and 31 with $\frac{1}{40}$ c.c., while all of them reacted with $\frac{1}{10}$. Of 92 cases in which there was no suspicion of cancer none showed a positive reaction in the presence of $\frac{1}{10}$ c.c. of serum, while with $\frac{2}{10}$ fixation was marked in only 9, *i. e.*, in cases of syphilis, tuberculosis, prostatic hypertrophy,

struma, cholelithiasis, pregnancy, and in 1 apparently normal individual. It is interesting to note that in the negative series there were fifteen syphilitics, all of which gave a positive Wassermann reaction. Positive reactions were obtained in both carcinoma and sarcoma. Should v. Dungern's results be confirmed by other investigators, an important step would indeed have been taken toward an earlier diagnosis of malignant disease. The few investigators who have worked with his earlier technique do not seem to have been so successful as he himself claims to have been. (See also p. 174.)

Protective Ferments have been demonstrated in the blood in a large percentage of cases.

The Gastric Juice.—While the study of the gastric juice will be taken up in detail in connection with the consideration of cancer of the stomach, it may briefly be stated at this place that the cancerous process *per se*, irrespective of the seat of the disease, very commonly leads to impairment of the secretion of hydrochloric acid or to its complete suppression. This observation was first made by Fenwick, and has since been confirmed by various investigators (Ewald, Riegel, Moore-Alexander, Kelly and Roaf, Friedenwald and Rosenthal). The latter observers examined 29 cases of cancer other than of the stomach; of these, 9 showed a low total acidity (10 to 27), with entire absence of free hydrochloric acid; 10, a low total acidity (32 to 52), with a marked diminution of free acid (0.024 to 0.092 per cent.), and the remaining 10, a normal acidity with a normal percentage of free acid. In other words, there was a deficient secretion of acid in 65 per cent. of the cases. The same observers made the interesting observation that this absence or diminution of acid persists even after the complete removal of the cancerous mass. Of ten cases studied in this direction the acid did not return where it had been absent, nor did it increase in those cases where it had been diminished.

The Urine.—The urine shows no characteristics which can be attributed specifically to the cancerous progress. In accordance with the marked loss of body tissue there is, of course, sooner or later, excessive nitrogenous katabolism. In some cases this occurs early, in others, late; in some it pursues a rapid, in others a slow course. According to Robin there is a marked demineralization, while the individual examination may suggest a retention. The elimination of oxyacids and of aromatic substances is at the same time increased. Glucosuria does not belong to the urinary picture of cancer *per se*, but may occur in special cases (see Cancer of Pancreas). Small amounts of albumin may be met with temporarily, but are unimportant. In two isolated instances of carcinomatosis involving the bones (secondary to carcinoma of the breast and of the stomach respectively) Bence-Jones' protein has been found in the urine.

CANCER OF THE BREAST

The laboratory findings in cancer of the breast are essentially those which have already been considered in the section on Cancer in general. Leukocytosis is variable, depending to a great extent upon the existence of metastasis.

CANCER OF THE INTESTINE

Essential Factors.—The blood changes are the same as in cancer of the stomach; in addition there may be symptoms on the part of the feces which deserve consideration, notably the presence of blood in macroscopic or occult amount and the presence of tumor particles.

The Blood.—The blood shows no special features which distinguish cancer of the bowel from cancer of the stomach. Here as there we meet with a tendency to secondary anemia of the chlorotic type which is frequently obscured to a greater or less extent by a relative polycythemia, and here as there leukocytosis is variable.

In doubtful cases some of the serological methods of diagnosis should be tried, more especially the meiostagmin test and the estimation of antitrypsin. (See Cancer.)

The Stomach Contents.—These likewise show no characteristic features. Free hydrochloric acid may be absent no matter where the tumor is situated. In duodenal cancer lactic acid and Boas-Oppler bacilli may be found, especially when obstruction has become marked. When stenosis of the descending or transverse portion of the colon or of the beginning of the jejunum exists, bile and pancreatic juice are commonly encountered.

The Feces.—In cancer involving the region of the sigmoid the stools are sometimes fluid and discharged in small quantities at a time. Ribbon-shaped masses, pencil-like stools, or small round balls resembling the excrements of sheep are seen in other cases involving the lower portion of the large intestine, and are especially common in cancer of the rectum; they are not pathognomonic of an organic stenosis, however, as was once supposed. In advanced cases of cancer of the lower tract the odor is sometimes very offensive, and naked eye examination may reveal the presence of blood and pus. Careful search may lead to the discovery of fragments of the tumor. The presence of blood, even though this be not demonstrable macroscopically, can be made out either by microscopic or chemical examination, in most cases quite early in the course of the disease. The higher up the seat of the lesion the less apt is one to find blood with the microscope, while intact corpuscles can be found when the disease has attacked the descending colon or rectum. The extent of the bleeding is variable. In some cases alarming hemorrhages may be observed, while in others the bleeding is occult and can only be dem-

onstrated by chemical examination. Between these extremes there are all gradations. The color of the blood depends to a great extent upon the seat of the lesion; the nearer to the anus the brighter its color; the further removed the darker will it appear; and in cancer of the upper portion of the small intestine the feces may be black like tar. This is the more apt to be the case the longer the time that has elapsed since the bleeding occurred. If this is copious and diarrhea exists the color may be red, even though the hemorrhage has occurred in the duodenum; more commonly the color is dark.

The Urine.—The urine presents no special features which require consideration beyond the fact that the amount of indican and the conjugate sulphates in general are much increased in obstruction, involving the small intestine, while corresponding involvement of the large intestine does not produce this effect. Whether or not this rule is an absolute one remains to be seen. In cases of duodenal cancer involving the common duct the various bile constituents will, of course, appear in the urine

CANCER OF THE KIDNEY

Here also the general findings are the same as those which are met with in cancer, involving other organs. (See Cancer.) There is, however, a marked tendency to hyperleukocytosis which is less common in primary cancer elsewhere. Of 10 cases recorded by Cabot, the count exceeded 10,000 in 7; in 6 of these it was higher than 20 000, in 2 it exceeded 40,000, and in 1 it was above 80,000. Von Limbeck mentions a case in which the leukocytes steadily rose from 18,514 to 80,541. It is noteworthy that among these cases also a high neutrophile va ue was associated with a persistence of eosinophiles in normal numbers. In one case I found the mast cells quite constantly between 3 and 5 per cent.

The Urine.—Hematuria is a common symptom in renal cancer, and is not infrequently the first to excite attention; it occurs in fully 50 per cent. of the cases. Sometimes the bleeding is only microscopic, while at others it may be copious, the blood appearing in clots; these may be long and cylindrical, being virtually casts of the ureter. The bleeding occurs either continuously or intermittently. Tumor particles are rarely found in renal cancer, while in cancer of the bladder they are common. In other respects the urine may show no abnormality.

CANCER OF THE LIVER

The general laboratory findings in cancer of the liver do not differ materially from those observed in cancer of other organs (see Cancer in general), excepting in so far as the cancerous process leads to jaundice and the consequent appearance of bile in the blood and the

urine. The degree of anemia, however, is usually more marked, and hyperleukocytosis more frequent (in over one-half of the cases).

The urine, even if not bile tinged, is highly colored and may contain a trace of albumin and hyalin and finely granular casts.

CANCER OF THE LUNG

For a consideration of the general findings see the section on Cancer. When the process involves the lungs the sputum may present features which are in a measure characteristic. It may be gelatinous and colored red, but more frequently it has a prune-juice appearance; more rarely it is grass-green or olive-green in color. At times no sputum is obtained. In suspected cases a careful search should be made for tumor particles.

CANCER OF THE ŒSOPHAGUS

The laboratory findings are essentially those which have been considered in the general section on Cancer. It is to be noted in particular that the red count and hemoglobin values are apt to be high, notwithstanding the very evident existence of severe anemia. This, unquestionably, is due to the great concentration of the blood which develops in such cases, and which is further evidenced by the high content of solids (26.5 and 27.3 per cent., v. Noorden). The leukocytes are usually not increased, and there may, indeed, be leukopenia. Occasionally bits of the tumor may be brought up in the eye of the stomach tube.

CANCER OF THE PANCREAS

Essential Factors.—Severe secondary anemia with relative polycythemia; irregular hyperleukocytosis; cholemia; steatorrhea and azotorrhea; diminution in the amount of fecal diastase; irregular glucosuria; severe choluria; irregular ascites.

The Blood.—The blood picture in cancer of the pancreas is that of cancer in general (which see), and is not influenced to any noticeable extent by the localization of the lesion. The cachexia, however, develops with special rapidity. Hyperleukocytosis is fairly common, occurring in fully 75 per cent. of the cases; the figures, however, are not very high, varying between 10,000 and 20,000.

Since in the majority of cases the disease involves the head of the organ, jaundice is a common and early symptom, the presence of bile being demonstrable in the serum. When once it has appeared it is not apt to disappear, but continues to the end, steadily increasing in intensity (black jaundice).

For a consideration of the serum reactions see the general section on Cancer.

The Feces.—Steatorrhea and azotorrhea are common symptoms in pancreatic cancer, and may be seen in extreme form in those cases which are associated with jaundice. The amount of fecal diastase is quite constantly diminished.

The Urine.—The general urinary changes are the same as those which occur in cancer of the stomach. The claims which Cammidge set up for the diagnostic value of the reaction which bears his name have not been supported, and it would hence be unwise to lay stress upon it any longer.

Glucosuria is a fairly common symptom of cancer of the pancreas, but usually does not appear until the destruction of the gland is far advanced; it was noted in 13 of 50 cases collected by Mirallie. Very curiously the condition may disappear before death.

Choluria is very frequent owing to the common involvement of the head of the pancreas and consequent jaundice. When once it has appeared it continues to the end. Albuminuria and cylindruria are then apt to develop. Hyunhouser speaks of the presence of large amounts of diastase; this, however, seems more frequent in acute disease of the pancreas than in chronic cases.

Ascites.—Ascites occurs in about one-ninth of the cases, owing to compression of the portal vein. Exceptionally chylous ascites has been observed, presumably due to rupture of the thoracic duct.

CANCER OF THE PERITONEUM

As peritoneal carcinomatosis is almost always secondary to cancer of other organs, the laboratory findings will be those referable to cancer in general, modified more or less by the special site of the primary lesion. Hyperleukocytosis is common, viz., in 15 of Cabot's 23 cases. This is due to an increase of the neutrophiles, but contrarily to what we see in bacterial sepsis the eosinophiles are quite constantly present in normal or even in maximal normal numbers. In some cases the polynucleosis is quite marked, even though the total number of the leukocytes is not increased. Occasionally very high figures are obtained, which are exceeded only in leukemia; Cabot thus mentions a count of 152,000.

When ascites exists a careful examination of the morphological elements may reveal the presence of cancer cells. These may be indistinguishable from normal endothelial cells; the latter, however, rarely give the glycogen reaction which is common in tumor cells, and these, moreover, may show mitoses which are never found in non-malignant exudates. Commonly the mitoses are atypical; the division of the nucleus is not followed by a division of the cell; the chromosomes are short and show no polar or equatorial arrangement. Occasionally bits of the tumor may be found in the sediment or occluding the eye of the trocar.

Quincke has drawn attention to the occurrence of large numbers of fat droplets in malignant exudates, which may attain a diameter of 40 to 50μ. At other times, however, they are so small and so numerous as to give a chylous (milky) appearance to the exudate. A similar appearance may be produced by the presence of albuminous granules, which may be distinguished from fat by their insolubility in ether and the fact that they are not stained with the common fat dyes, such as Sudan, scarlet R, and alkanin. The occurrence of numerous fatty acid crystals should also excite suspicion of a neoplasm.

When jaundice exists the ascitic fluid is bile-tinged; frequently also it is hemorrhagic, and following intraperitoneal hemorrhages pure blood may be found. The amount may be very large, frequently exceeding 5 liters, and rapidly accumulates after tapping. According to Bard and Milian the fluid is hemolytic for normal red cells, which corresponds to what we would expect considering the hemolytic property of the blood serum (see Cancer—Serology).

CANCER OF THE STOMACH

Essential Factors.—Secondary chlorotic anemia with relative polycythemia; hyperleukocytosis in advanced cases with metastases; positive meiostagmin reaction and increased serum content in antitrypsin; presence of isohemolysins; absence of free hydrochloric acid; presence of lactic acid; decrease or absence of the gastric ferments and their zymogens; presence of the Boas-Oppler bacillus; occult blood in the stomach contents and feces; low chloride values in the urine; increased indicanuria.

The Blood.—*The Red Cells and Hemoglobin.*—I have pointed out in the general section on cancer that anemia, usually of the chlorotic type, develops sooner or later in all cases. The extent of the corpuscular anemia may at first be masked by a general concentration of the blood, however, so that it is not uncommon to find the red cells but little below 4,000,000, even though a tumor be already palpable. This relative polycythemia is especially marked in cancer of the internal organs, and especially in cancer involving the digestive tract. In cancer of the esophagus and of the stomach, when involving the pylorus, normal and even supernormal values are frequently obtained, owing to a defective absorption of water and frequent vomiting. Taking the series reported by Cabot from the Massachusetts hospital and the Hopkins series mentioned by Emerson together, there are 263 cases. In 60 of these the count was 5,000,000 or more, and in only 42 was it below 3,000,000; the average was about 4,000,000. Sooner or later, as cachexia develops, he relative polycythemia diminishes, but even then the red count rarely expresses the actual state of the anemia. While this is the rule, there are not

infrequent exceptions, where the patient quite early in the disease already becomes markedly anemic, and where the count actually shows a rapidly progressing and extensive loss of red cells. In these cases the color index may not be diminished; even if not increased, it is frequently normal. It is remarkable that in many of these severely anemic cases, with normal or increased indices, the local lesion may be quite small, and in the absence of a palpable tumor it is not surprising that they are frequently mistaken for pernicious anemia. In some, indeed, the diagnosis is not made until after death. I have pointed out that a count below 1,000,000 usually points to pernicious anemia, but there are exceptions to this rule on both sides.

The morphological changes of the red cells have already been considered; their extent is, generally speaking, proportionate to the intensity of the anemia. Normoblasts are frequently present, though their number is usually small; megaloblasts are rare. (See Cancer.)

The Leukocytes.—The number of leukocytes is so inconstant in cancer of the stomach that no deductions of value can be drawn either from a normal number or from the existence of hyperleukocytosis. In Cabot's series of 235 cases they were increased in only 69, and of these, 27 had values below 15,000. It is to be noted, however, that Cabot excluded from this series all cases in which there was evidence of metastasis. In the 19 cases in which metastases were manifestly present hyperleukocytosis was noted in 15, the numbers varying between 10,000 and 105,600. The last figure was obtained in a case in which "a cancer of the stomach with metastases in the liver perforated into the peritoneal cavity and started a virulent, quickly fatal peritonitis."

The differential count usually shows no essential deviations from the normal, unless hyperleukocytosis exists, when the neutrophiles are correspondingly increased, with sometimes a persistence of the eosinophiles. (See Cancer.)

Digestive Leukocytosis.—According to most writers absence of digestive leukocytosis is noted in from 80 to 90 per cent. of all cases of cancer of the stomach, and it was once thought that such an occurrence in doubtful cases constituted an important factor in the diagnosis of malignant disease. Generally speaking this is true. The symptom, however, is not pathognomonic. It is usually absent in cases of cancer showing a hyperleukocytosis, and has occasionally been noted in some non-cancerous cases. As a corroborative symptom it is nevertheless of a certain value.

Serology.—(See the general section on Cancer.)

The Gastric Contents.—GENERAL CHARACTERISTICS. — *Amount.* — The amount of material which may be found in the stomach one hour after the ingestion of Ewald's test breakfast depends upon the motor power and the existence or non-existence of a pyloric stenosis. When no obstruction exists and the motor power is good, as is

not infrequently the case early in the disease, normal quantities will be found, and it will be observed that remnants of previous meals are absent. With the development of a stenosis, however, and in proportion to the loss of motor power, the amount increases, and it is then common to meet with food remnants in the fasting organ. Average figures are here of no importance. When the stenosis is of high grade a liter or more may be withdrawn.

Blood.—In advanced cases, in consequence of the gross admixture of blood, the stomach contents may present the color of weak coffee. Smaller hemorrhages frequently occur much earlier, but may not affect the general appearance; the blood may, indeed, not be demonstrable on microscopic examination. Adequate chemical examination, however, will frequently reveal its presence, both in the stomach contents and in the feces; its presence in the latter is especially significant. In a series of 150 cases, Osler and McCrae noted blood macroscopically in the vomit in 21.8 per cent., while in the feces it was found in the occult form in all cases (see below). In Friedenwald's series of 733 cases visible blood was noted in only 7.5 per cent. Coffee-ground contents were obtained in 61.8 per cent.

Fermentability.—The fermentability of the stomach contents, even in cases where the pylorus is not involved, is according to Strauss, relatively increased, and out of proportion to the amount of residue from the test breakfast.

Tumor Particles.—In advanced cases a careful examination of the contents and washings may lead to the discovery of tumor particles; these should be placed in 10 per cent. formalin and submitted to histological examination. For the early diagnosis, of course, their discovery is of no importance; their presence, however, is more common than is generally believed.

Chemical Examination.—*Absence of Free Hydrochloric Acid.*—In most cases (80 to 90 per cent.) of cancer of the stomach chemical examination reveals the *absence of free hydrochloric acid,* at a time when the patient first seeks medical advice. This was first established by v. d. Velden, and has since been abundantly confirmed. At one time there was a tendency to view anachlorhydria as pathognomonic of cancer of the stomach. This idea has been abandoned, however, as it has been shown that cases of cancer of the stomach may occur in which hydrochloric acid is not only present, but present in excessive amounts. This is true especially of those cases in which the malignant growth has started upon the base of an old ulcer. It is noteworthy, moreover, that in early cases of cancer, even in the absence of ulcer, hydrochloric acid may at times be demonstrable and then disappear for days and weeks and subsequently reappear. It has been shown, furthermore, that anachlorhydria may occur in other conditions which have nothing to do with cancer, such as gastric anadeny, toxic gastritis, atrophic catarrh, certain types of nervous

dyspepsia, and occasionally also in cases of phthisis, heart disease, various febrile conditions, etc. I have pointed out in the general section on Cancer that the same may be observed quite frequently in cancer of other organs in which the stomach is not directly implicated. The symptom is, nevertheless, of value, but must be studied in its relation to the other symptoms. Regarding the stage of the disease at which free hydrochloric acid first disappears, this seems to occur already quite early, but it may temporarily reappear, to disappear again, and so on. This irregularity in the secretion of hydrochloric acid in a patient within the cancer age should always excite attention, particularly if such an individual has previously enjoyed good digestion and then becomes dyspeptic fairly acutely.

In Friedenwald's series of 733 cases in which the stomach contents were examined entire absence of hydrochloric acid was noted in 89 per cent., hypochlorhydria in 2.8, hyperchlorhydria in 4.1 and normal values in 3.1. (Compare herewith the findings in ulcer of the stomach.)

While free hydrochloric acid is usually absent in cancer of the stomach, this does not mean that no hydrochloric acid is secreted. This may, indeed, occur; in many cases, however, its formation has not ceased, but is merely impaired, so that an amount is secreted which is insufficient to satisfy the affinities of the test meal and then to appear in the free state. The degree of this insufficiency is readily ascertained by titrating the stomach contents with decinormal hydrochloric acid to the point where this appears in the free form.

Presence of Lactic Acid.—Absence of free hydrochloric acid in gastric cancer is almost always associated with the presence of notable amounts of lactic acid (0.1 to 0.4 per cent.); a definite motor insufficiency, however, seems of paramount importance for its production. Absence of lactic acid is rare in cases where these two factors co-exist; when this is observed it will probably always be found that albuminous digestion has not yet been seriously interfered with, and that the secretion of hydrochloric acid, while not sufficient to cause its appearance in the free state, suffices, nevertheless, to satisfy most of the albuminous affinities of the test meal. The amount of hydrochloric acid in such cases is manifestly sufficient to impede the formation of lactic acid. When large amounts of hydrochloric acid occur, as in those cases of cancer which develop upon the basis of an ulcer, lactic acid is never present. In non-cancerous cases the appearance of lactic acid in notable amounts is rare; it has been exceptionally observed in benign stenoses and atrophic catarrh with atony, but this hardly lessens the diagnostic value of the symptom in cancer. It is not pathognomonic, to be sure, but it is very significant. In Friedenwald's series of 733 cases lactic acid was present in 81.9 per cent. of the cases.

Regarding the stage of the disease at which lactic acid can first

be demonstrated in considerable amount, it appears that this may occur quite early, and that at such a time periods of chlorhydria and lactic acid production may alternate. In doubtful cases such an occurrence would, in my judgment, be a sufficient basis for recommending an exploratory laparotomy.

The Ferments and Their Pro-enzymes.—In those cases in which the production of hydrochloric acid is seriously impaired the gastric ferments also will be found much diminished or absent. In some cases the pro-enzymes may then still be demonstrable, while in others their formation also has ceased.

Microscopic Examination.—When lactic acid can be demonstrated on chemical examination, the microscope will show the presence of the so-called Boas-Oppler bacilli, their number being in a general way proportionate to the amount of acid present. These organisms are strong lactic acid producers themselves, and in advanced cases frequently crowd out all the rest. Their demonstration has the same significance as the chemical proof of the presence of lactic acid. They should be sought for in the first fluid which is withdrawn, previous to washing out the stomach. In Friedenwald's series of 733 cases the bacilli were present in 79.3 per cent., in all of which lactic acid was present.

Sarcinæ may be encountered in incipient cases of pyloric cancer, so long as hydrochloric acid is secreted; in advanced cases they are hardly ever seen. Oppler was unable to find them twenty-four hours after their introduction in pure culture and in large numbers. Friedenwald found them in only 3.2 per cent. of his cases.

Isolated *yeast cells* may at times be seen; their presence hardly ever leads to abundant gas formation; this is peculiar, since the organisms will develop in large numbers in the stomach contents of cancer patients after removal from the body.

Protozoa have been found in the stomach contents of cancer patients by several observers (Cohnheim, Nichols). The organisms in question are for the most part the Trichomonas intestinalis and Megastoma entericum. Their growth, no doubt, is rendered possible by the alkaline reaction of the cancer juice, which exudes from various crevices of the diseased mucous membrane.

Pus is rarely found in large amount, while leukocytes are usually present in fair numbers in advanced cases. The presence of red cells likewise is common.

The Feces.—It is noteworthy that occult blood may be demonstrated in the feces in practically every case of cancer of the stomach, and that it is usually present at a time when the patient first seeks the advice of a physician. In Friedenwald's series of 216 *early* cases, 201 (93 per cent.) showed occult blood, while in the entire series of 642 positive findings were obtained in 594 (92.5 per cent.). Tar-colored stools were noted in 18.9 per cent.

In the diagnosis between ulcer and cancer, intermittent bleeding points to ulcer and continuous bleeding to cancer; this, however, is not an invariable rule, since ulcer cases occur in which a positive result may be obtained at every examination, the amount gradually diminishing and finally disappearing as recovery occurs.

The Urine.—In early cases of cancer there may be no urinary changes whatever, but as the disease progresses various deviations from the normal occur. Owing to insufficient absorption of water, the amount of urine is frequently much reduced, 400 to 500 c.c. per diem being common values. The color is high and the specific gravity increased. In cancer of the pylorus, corresponding to the non-production or deficient production of hydrochloric acid, there is no diminution in the curve of acidity after meals. The chlorides are markedly diminished in most cases, which is what one would expect considering the diminished appetite of the patient. Possibly there is also an increased retention. In many cases the daily output is less than 2 grams.

Through the studies of F. Müller and Klemperer it has been established that in carcinoma the nitrogenous katabolism is increased. The elimination of ammonia is at the same time relatively high, corresponding to 8 to 12 per cent. of the total nitrogen.

The indican is usually much increased in advanced cases, and in these Rosenbach's reaction is also demonstrable. Regarding the time when the increased indicanuria first makes its appearance there are no reliable data, but my own impression has been that it usually exists already when the patient first seeks medical advice.

Transitory albuminuria is not uncommon, but insignificant; albumoses also have been repeatedly observed, while the earlier reports on the presence of peptone are inaccurate, the findings probably having reference to albumoses.

Acetone, diacetic acid, and oxybutyric acid have likewise been encountered; regarding the frequency of such an occurrence, however, no data exist. The high ammonia values, which are relatively common, would suggest that acidosis also may be common.

According to Leo, Hoffmann, and Stadelmann the urinary pepsin is much diminished.

CANCER OF THE UTERUS

In cancer of the uterus, owing to the frequent occurrence of hemorrhages, anemia commonly develops earlier and is apt to become more intense than in the other forms of cancer. Hyperleukocytosis also is more common, and may be referable in part to the frequently associated septic infections. Early diagnosis rests upon the early excision of pieces of tissue and their histological examination. When the disease is advanced tumor particles may be found in the vaginal discharge.

CEREBRAL HEMORRHAGE

Essential Factors.—Hyperleukocytosis (neutrophilic); transitory albuminuria; irregular presence of blood in the cerebrospinal fluid and of macrophages containing red cells or hematoidin.

The Blood.—Hyperleukocytosis occurs in the vast majority of cases. In Cabot's series of 51 cases a count higher than 10,000 was noted in 45, and one above 15,000 in 22. The increase is usually of the neutrophilic type, but in some cases a lymphocytosis has been noted.

The Urine.—The urinary picture will largely depend upon the existence or absence of associated or complicating factors. A number of observers have described the occurrence of a transitory albuminuria in connection with the attack, and this no doubt is quite common. Transitory glucosuria, on the other hand, seems to be exceptional. Von Jaksch was thus unable to demonstrate the presence of sugar in any of 50 recent cases of hemiplegia.

The Cerebrospinal Fluid.—In cases of hemorrhage into the ventricles pure blood is obtained, while such a result is, of course, a mechanical impossibility in cases of epidural hematoma. In subdural hematoma, on the other hand, blood may also find its way into the subarachnoid space, but the amount is always small, and cannot be compared with that seen in cases of ventricular hemorrhage. Whenever, then, as in traumatic cases with severe cerebral symptoms, the surgeon is confronted with the question whether or not to trephine, lumbar puncture may furnish much valuable information. If in such cases no blood at all is found, it may be inferred that an epidural hematoma or a subdural hematoma of slight extent only exists; an operation may then be performed. If, however, pure blood is encountered it would be justifiable to assume the existence of extensive injury to the brain substance proper, or, in cases in which the history is obscure, an intracerebral hemorrhage with rupture into the ventricles. In such cases the idea of an operation would, of course, be entertained only under exceptional conditions. If, further, the fluid is only tinged with blood, a subdural hematoma probably exists, and an operation should be advised. Accidental hemorrhage—viz., hemorrhage referable to the puncture itself—can be readily recognized, as the first few drops only are then tinged with blood, or the blood appears only after the flow has been definitely established; the amount, moreover, is insignificant.

In connection with cerebral hemorrhage (especially hemorrhage into the ventricles) Sabrazès and Muratet have described large, round, oval, or polyhedral cells, occurring either singly or in plaques, each provided with a single oval nucleus containing several nucleoli. These cells commonly contain red blood corpuscles, as also crystals and amorphous particles of hematoidin, leukocytic

nuclear debris, and vacuoles. They are macrophages, derived undoubtedly from the endothelial lining of the subarachnoid spaces. Besides, granular structures may be met with which contain globules of fat, nuclear debris, globules of myelin, red cells, and blood pigment. What these latter cells are is not known. Sabrazès inclines to the view that they are neuroglia cells.

CHALICOSIS

In chalicosis silicates are found in the sputa.

CHICKENPOX (VARICELLA)

The Blood.—The absolute leukocyte count is not materially affected in chickenpox. The results of the differential count seem to depend somewhat upon the stage of the disease, but are apparently not constant. Some observers report a moderate increase of the neutrophiles with absence of the eosinophiles during the stage of active pustulation, while others speak of an increase of the large mononuclears. During convalescence the eosinophiles may be increased.

Ordinary chickenpox has no material effect upon the *red corpuscles* and the *hemoglobin*, but in cases of the necrotic and hemorrhagic form a certain degree of secondary anemia may be observed.

The Urine.—Urinary changes are uncommon, but in some instances nephritis may develop with corresponding albuminuria and cylindruria.

CHLORO-ANEMIA

The term chloro-anemia has been suggested to designate certain cases of anemia in which the blood picture of chlorosis is associated with evidence of a secondary degeneration of the red cells (anisocytosis, poikilocytosis) and in some cases of anemic regeneration (polychromasis, granular "degeneration").

CHLOROMA

Essential Factors.—Marked secondary anemia; irregular hyperleukocytosis with lymphocytosis predominating; irregular myelocytosis; irregular increase of mast cells and eosinophiles.

The Blood.—*The Red Cells and Hemoglobin.*—In all cases of chloroma there is an actively progressive loss of red cells and hemoglobin, such as we are accustomed to find in acute lymphatic leukemia. The oligocythemia may become as extensive as in pernicious anemia. Pope and Reynolds have reported a case in which the count dropped from 2,450,000 to 900,000 and the hemoglobin from 45 to 25 per cent., in nine days. Butler mentions an instance in which death

occurred about seven weeks after the onset of the first symptoms, with a red count of only 300,000. The color index is somewhat variable, but usually not increased. The morphological changes in the red cells are those of a secondary anemia, and the nucleated red cells which may be present in moderate number are practically all of the normoblastic type.

The Plaques.—The blood plates may be increased.

The Leukocytes.—The leukocyte count is extremely variable, and it is possible, as has been suggested by several writers, to distinguish between an aleukemic and a leukemic form of the disease. The former is unquestionably the more common. Bromwell has reported a case in which the white count was only 8000. More often there is a moderate hyperleukocytosis of from 15,000 to 50,000 cells. The cases with a definitely leukemic increase are in the minority. In a case described by Sternberg the count was 100,000; in another recorded by Pope and Reynolds it rose to 360,000. The differential findings are likewise variable. In the majority of cases there is a lymphocytosis, either of the small- or the large-cell type, which commonly amounts to from 80 to 90 per cent. of the total number. In others, myelocytes enter prominently into the foreground, so that one may speak of a lymphadenoid blood picture on the one hand and a myeloid picture on the other. In one case reported by Benjamin and Sluka the myelocytes ranged between 37 and 57 per cent.; and the total number of the leukocytes between 7000 and 26,000. The type of the predominating myelocyte in these cases is usually, however, not the one which controls the blood picture in the common form of myelocytic leukemia, but is generally described as "atypical;" the cell is essentially a large mononuclear cell with a large feebly staining nucleus and a basophilic protoplasm which may or may not contain a variable number of blue granules of irregular size and at times some that are finer and show a somewhat purplish tone (myeloblasts). These cells are variously designated as atypical myelocytes, promyelocytes, myelocytes of Blumenthal, etc. They are unquestionably juvenile cells, which probably stand somewhere between the original mother cell of all leukocytes (macrolymphocyte, lymphoid cell) and the neutrophilic myelocytes proper. Sternberg, in his case, mentions the occurrence of numerous cells of the type of the large mononuclear leukocyte (85 per cent.) which on staining with Ehrlich's triacid stain could be shown to contain neutrophilic granules, but which, nevertheless, so far as size, form and distinction of the granules was concerned, did not correspond to the usual neutrophilic myelocytic type. He regards these as aberrant types and as the expression of a sarcomatous process, a view which is vigorously combated by Pappenheim.

The other granular leukocytes of the blood are variable in their behavior. Some writers have reported an increase of the eosino-

philes and mast cells similar to what is seen in ordinary myelocytic leukemia, while others speak of a decrease or even an absence of these elements. In a few cases the blood picture is a mixed one, there being both lymphocytosis and myelocytosis. Klein and Steinhaus have reported a case of this order, in which the lymphocytes varied between 50 and 66 per cent. (47 per cent. of macrolymphocytes) and the myelocytes from 16 to 32 per cent. (total leukocyte count 20,000 to 41,000).

The Urine.—The urinary picture in chloroma is essentially the same as that which is seen in acute lymphatic leukemia (which see).

CHLOROSIS

Essential Factors.—Oligochromemia without a corresponding oligocythemia; low color index; large mononucleosis.

The Blood.—*The Red Cells and Hemoglobin.*—Chlorotic anemia is characterized by the existence of an oligochromemia which is out of all proportion to the corresponding oligocythemia. In some cases, indeed, the red cells are not diminished at all. Graeber's average count was 4,482,000, with 5,700,000 and 3,805,000 as maximal and minimal values. Cabot's average of 192 cases was 4,052,000; Thayer's, 4,096,544; and Da Costa's, 3,876,000. Occasionally very low values are met with, which in many cases, no doubt, are due to some secondary factor, such as hemorrhages from the stomach or uterus, but it appears that in exceptional cases the chlorotic process *per se* can give rise to severe corpuscular anemia. Hayem mentions minimal values of 2,500,000; Cabot gives 1,932,000; Thayer, 1,953,000; and Da Costa, 1,720,000.

As average of 94 cases I found a hemoglobin value of 42.5 per cent.; with 17.5 as minimal figure; Cabot's average was 40.4; Thayer's, 42.3; and Da Costa's, 41.3.

The color index accordingly is low—0.5, as average in Cabot's series; occasionally it may fall below 0.3. Corresponding to this low color index, morphological examination shows a decided increase in the size of the central pale area of the red corpuscles (*central anachromia*), and in marked cases the so-called pessary forms control the entire blood picture. Some writers maintain that the average size of the cells is diminished; others do not corroborate this, but find material deviations in both directions, with approximately average normal values. Macrocytes may be encountered, but they do not control the picture; microcytes, on the other hand, are more common. Poikilocytosis may be marked in severe cases, but in the average case deviations in size are more frequent than deviations in form. Exceptionally the blood picture may resemble that of pernicious anemia. Polychromatophilia of the red cells is relatively common, while stiple cells are usually rare; sometimes, however, they may be numerous.

I have recently observed a case of this kind, where a dozen or more could be seen in every field ($\frac{1}{12}$). Nucleated red cells (normoblasts), in contradistinction to what we see in the secondary anemia of malignant disease, are very scarce, so that a long search may be necessary before one is found; megaloblasts are a great rarity.

The Leukocytes.—The leukocytes are not increased in uncomplicated cases, and may, indeed, be diminished; this is especially apt to occur in the severer forms. Cabot's average count was 7400; Thayer's, 7485; and Da Costa's, 6457. Da Costa's minimal count was 800. Inflammatory and other complications may, of course, increase the number as in normal individuals. The differential count shows a remarkable increase of the large mononuclear cells in a large proportion of cases. Da Costa found this in 30 out of 37 cases, the values ranging between 2 and 40 per cent., while the small lymphocytes are only exceptionally and then only slightly increased. Some other writers speak broadly of an increase of the lymphocytes, but manifestly have reference to the large mononuclears and small lymphocytes collectively. Corresponding to a general increase of the mononuclear forms, we find in many cases minimal normal values of the neutrophiles. The eosinophiles are diminished in almost all cases, and may, indeed, be absent (in 70 per cent. of Da Costa's cases). A few neutrophilic myelocytes may at times be found in some of the graver cases.

The Plaques.—The plaques are usually very much increased.

General Characteristics.—The blood as it exudes from the puncture is pale, the degree of pallor corresponding to what we would expect from the appearance of the patient. This is in distinct contrast to what is frequently seen in various types of secondary anemia, associated with marked loss of fluid from the body, where, owing to a concentration of the blood, there is a relative polycythemia and accordingly a relative increase in the amount of coloring matter. As Grawitz expresses it, we have in chlorosis a state of polyplasmia, as contrasted with one of oligoplasmia.

The coagulability of the blood is not diminished; if at all altered, it is increased, contrasting markedly with what is found in pernicious anemia and leukemia.

The specific gravity of the blood, in accordance with the existing polyplasmia, is diminished, usually varying between 1.035 and 1.045 while the disease is active; a further decrease is suggestive of complications (Grawitz). The solids are correspondingly low—13 to 16 per cent. (Grawitz). Regarding the alkalinity of the blood there are no data which have been obtained with satisfactory methods.

The iron content is diminished, corresponding to 0.019 to 0.04 per cent. by weight; the serum itself is free from iron.

The Urine.—There are no essential deviations from the normal.

CHOLANGITIS AND CHOLECYSTITIS

Essential Factors.—Cholemia; hyperleukocytosis with septic factor; delayed coagulation; irregular bacteriuria; bacteriasis of the gall-bladder; presence of gallstones; choluria.

The Blood.—*The Red Cells and Hemoglobin.*—The blood picture, so far as the red cells and hemoglobin are concerned, depends to a great extent upon the underlying condition. If this is a systemic infection or a cancerous condition involving the common duct, there will usually be more or less anemia at the outset. If, however, as is more frequently the case, the inflammation of the gall-bladder and the bile passages is the outcome of the existence of gallstones, there need be no deviation from the normal at the start. Subsequently, of course, anemia is a necessary consequence of the infection. Frequently this does not become numerically very evident, owing to concentration of the blood. In cholangitis it may be obscured, so far as the appearance of the patient goes, by the existing jaundice. In uncomplicated cases of cholecystitis, on the other hand, this does not occur; its development here always indicates an extension of the disease to the liver. In cholangitis absence of jaundice is rare. The bile pigment can readily be demonstrated in a centrifugalized specimen of the patient's blood, when the serum will present a golden-yellow color.

The Leukocytes.—In *catarrhal cases* the leukocyte count need not be increased; usually it is normal. When *suppuration* develops, however, marked hyperleukocytosis is the rule. The only exceptions probably are those cases in which the patient is overwhelmed by the toxemia from the start. Emerson mentions such a case (cholangitis) which proved fatal, in which with a temperature of 106° the absolute count was only 6400. Frequently the counts are very high (45,000 to 50,000), while the average ranges between 20,000 and 30,000, with 12,000 to 15,000 in the milder cases (cholecystitis). In cholecystitis there is a return to normal as the condition becomes chronic. The differential count shows the septic factor, which is demonstrable even in those very severe cases in which no absolute hyperleukocytosis develops.

The Coagulability.—The coagulability of the blood in those cases which are associated with jaundice is much delayed, a fact which must be borne in mind when operative interference is contemplated.

Bacteriological Examination.—This may reveal the presence of the offending organism. In other cases the organism causing the systemic infection is not identical with that found in the gall-bladder, the assumption being that the primary infection has prepared the soil for a secondary invader. Even in those cases in which the cholecystitis or cholangitis develops from local mechanical causes the bacterial invasion is regarded as secondary.

Contents of the Gall-bladder.—The contents of the gall-bladder are turbid, fibrinopurulent, bile-stained, and sometimes hemorrhagic. In fully 80 per cent. of the cases gallstones are found, which are now looked upon as having developed in consequence of a past infection. Among the bacteria which may be found may be mentioned the colon bacillus, Staphylococcus aureus, streptococci, the pneumococcus, the typhoid bacillus, the cholera bacillus, and as a preagonal invader the Bacillus lactis aërogenes.

Gastric Juice.—In cholecystitis a deficient secretion of hydrochloric acid is the rule. The same is found after extirpation of the gall-bladder or in connection with closure of the cystic duct or atrophy of the gall-bladder.

The Urine.—The urine contains biliary pigment in all those cases in which jaundice is marked, and when this has persisted for some time albuminuria and hyaline cylindruria develop; usually the amount is small. The other features of the urine are essentially those of an acute febrile process.

CHOLELITHIASIS

Essential Factors.—Irregular hyperleukocytosis of the neutrophilic type; delayed coagulation in the icteric cases; occurrence of the Cammidge reaction in cases of impaction in the pancreatic portion of the common duct or in the ampulla of Vater.

The Blood.—*The Red Cells and Hemoglobin.*—In many cases of cholelithiasis, where the attacks of colic occur only at rare intervals, the general health of the individual does not become impaired and there is accordingly no anemia. When the paroxysms become frequent, however, or when a stone becomes permanently impacted, resultant inflammatory and other complications develop (chronic icterus), and there is of necessity impairment of the general health. As the patients usually do not seek medical advice until the disease has reached this stage it will be understood why writers have noted anemia in a relatively large percentage of cases. Da Costa gives 30 per cent. as a conservative estimate of the average loss of hemoglobin and 15 per cent. as the average cellular decrease. These losses, it should be understood, are the outcome of complications and do not arise from the presence of stones in the bladder in itself. In many cases where marked anemia is noted this is the consequence of a septic condition or of hemorrhages referable to chronic jaundice.

The Leukocytes.—A simple attack of biliary colic does not necessarily cause a hyperleukocytosis, but this is, nevertheless, observed in some cases. In the Hopkins series of 36 cases (Emerson) the count rose to about 15,000. Aside from the periods of colic the count will depend upon the presence or absence of inflammatory complications. Of the 116 cases of Da Costa's series, 33 had a count exceeding 10,000. In Cabot's series of 50 cases the count was 10,000

or more in 20. In 6 the counts were made during attacks of colic, and in these the values varied between 5200 and 10,300, which is materially lower than the figures given by Emerson. A count higher than 18,800 was only noted in 3 (highest, 28,200); 2 of these were complicated by cholangitis (20,000 and 24,000 respectively).

The differential count shows normal values in those cases in which no hyperleukocytosis exists; when this is present the neutrophiles will be found increased, while the eosinophiles are diminished.

(For a consideration of the more important complications of cholelithiasis see these.)

Coagulation.—In cases which are complicated with jaundice the coagulation time is frequently much delayed, so that fatal bleeding may occur if the patients are operated upon at that time and without preliminary treatment with calcium salts. In Da Costa's series there was delayed clotting in 60 per cent. of the cases, the average time being eight and one-half minutes. In septic cases, on the other hand, the coagulation time is usually diminished, and fibrin formation correspondingly increased.

Chemical Examination.—In cases complicated by jaundice *chemical* examination of the serum will reveal the presence of bilirubin, which, moreover, can probably always be recognized with the naked eye.

Bacteriology.—Several investigators have reported positive findings on bacteriological examination of the blood. The examinations are most likely to yield results in cases marked by intermittent fever, during or immediately after the occurrence of a chill. The organisms encountered have been the colon bacillus, Staphylococcus aureus, streptococci, and the pneumococcus. In simple uncomplicated cases the findings will no doubt always be negative.

Gastric Juice.—During an attack there is commonly hypochlorhydria (see also Cholecystitis).

The Urine.—Urinary examination reveals no factors which could be referred to the presence of gallstones *per se.* When jaundice exists, the urine will naturally be icteric, and if the icterus be chronic a mild grade of albuminuria with hyaline cylindruria will sooner or later develop. Glucosuria, in my experience, is unusual. When it occurs it must be referable to an associated condition. As pancreatitis is frequently associated with and probably dependent upon the presence of gallstones in the common duct, the Cammidge reaction may be observed in such cases. Cammidge reports that in 30 of 51 cases of chronic pancreatitis a biliary calculus was found lodged in the pancreatic portion of the common duct or in the ampulla of Vater, and that the urine in all cases yielded typical crystals. My own rather limited experience has borne out the correctness of Cammidge's conclusions. The mere presence of stones in the gall-bladder, on the other hand, without involvement of the pancreas, does not give rise to the reaction.

CHOLERA ASIATICA

Essential Factors.—Relative polycythemia; hyperleukocytosis of the neutrophilic type with splenocytosis; presence of specific agglutinins; diarrhea; presence of the cholera vibrio in the feces; oliguria or anuria; increased ammonia content of the urine; presence of diamins.

The Blood.—*The Red Cells and Hemoglobin.*—In consequence of the rapid withdrawal of large quantities of fluid from the body there is a marked concentration of the cellular elements of the blood, and accordingly also an increase in the amount of hemoglobin. This may take place already a few hours after the onset of the disease. In the series of cases studied by Biernacki and Okladnysh values between 6,500,000 and 7,500,000 were common, while exceptionally 8,000,000 were counted.

The Leukocytes.—The concentration of the blood naturally also involves the leukocytes, but their increase usually exceeds that of the red cells, showing that the infection *per se* calls forth an increased production of cells. Generally speaking, the hyperleukocytosis is proportionate to the intensity of the infection, and ranges between 14,000 and 60,000, with 25,000 to 30,000 as average values. Biernacki states that all cases which in the algid stage show a leukocytosis of 40,000 to 60,000 soon prove fatal. A low count, on the other hand, does not necessarily mean that the patient will recover. Exceptionally in very mild cases, a hypoleukocytosis has been noted. The differential count shows an increase in the number of the neutrophiles, which rarely exceeds 80 per cent., however, owing to a simultaneous increase of the large mononuclears. According to Rogers this develops as the disease progresses, and becomes most marked toward the fatal end. He states that of 18 cases in which the number of the large mononuclears (normally 300 to 500) exceeded 2000, 14 died, while of 5 in which the count of these cells was lower than 2000, only 1 died. The eosinophiles are diminished, while the mast cells are increased in cases dying in the reactive stage (Sterrington).

General Characteristics.—The specific gravity of the blood is increased in consequence of the great loss of fluid; it has been found as high as 1.073. The alkalinity is materially diminished.

The Serum.—Examination of the serum shows the presence of specific agglutinins for the cholera vibrios, which may be demonstrated in some cases as early as the first day of active illness, and which persists into the fourth week following recovery (Achard and Bensaude). In doubtful cases this method of diagnosis should always be employed (see the technical part).

The Feces.—Diarrhea occurs in all cases. In the mildest types the patient has from three to eight stools in the twenty-four hours, and recovers without any severe symptoms. In the majority of cases, however, a premonitory diarrhea of moderate degree is fol-

lowed after one to three days by the cholera diarrhea proper, in which copious evacuations follow each other at brief intervals. In exceptionally severe cases the patient succumbs before a very active diarrhea has had time to develop (cholera sicca). Ordinarily the loss of fluid is very considerable, and may amount to 200 grams at each evacuation. At first the material is feculent, but it soon assumes the appearance of "rice water." The individual movements are then colorless, almost odorless, and on standing a finely granular, grayish-white sediment collects at the bottom. The reaction is neutral or alkaline. Chemical examination shows the presence of only 0.5 per cent. of solids, of which the greater portion is sodium chloride, with a little serum albumin. In severe cases blood may be present in variable amount. Microscopic examination reveals the presence of epithelial cells in various stages of degeneration, but many of them well preserved; also triple phosphate crystals, and numerous microörganisms. Among these the cholera vibrio may be demonstrated by adequate methods. To this end Dunbar's procedure may be recommended: A small flake of the suspected stool is placed upon two cover-glasses upon a drop of peptone solution and the one treated with a drop of normal serum and the other with a drop of cholera immune serum. On examining the specimens as hanging drops, it will be noted that the vibrios disappear already after a short while in the second specimen if they are the genuine cholera organisms. In this manner a positive result may be reached long before this is possible by culture (see also the technical part).

The Urine.—In nearly all cases of cholera the great loss of fluid through the intestinal tract leads to a high grade of oliguria and frequently to anuria, which often persists for several days. In one case of this kind the patient recovered even though no urine had been voided for fifteen days. When nephritis develops the urine becomes strongly albuminous, more so, in fact, than in other types of acute nephritis and coincidently large numbers of hyaline, granular, and epithelial casts are observed together with free epithelial cells, red blood corpuscles, leukocytes, and crystals of uric acid, calcium oxalate, or both. The macroscopic presence of blood, however, is unusual. Albuminuria also occurs in cases in which there is no actual nephritis, and with it casts and red blood corpuscles, but in such cases the urine promptly clears up within a week or two following the stage of reaction. During the active stage of the disease the urine is rich in indican and the conjugate sulphates are much increased both absolutely and relatively; the ammonia also is increased, as a rule, and with this diacetic acid may be demonstrated. Diamins also may be present in the urine, but especially in the feces. Brieger has shown that in cholera putrescin and cadaverin are unquestionably formed through the activity of the specific organism.

CHOREA

The Blood.—In uncomplicated cases of chorea examination of the blood does not show any essential deviation from the normal. Sometimes there is a mild degree of anemia, but in many other cases the red count and hemoglobin values remain unaffected. The leukocytes are not increased. Zappert and others speak of an increase of the eosinophiles.

The Urine.—The urine shows no special abnormalities.

In two cases of Huntington's chorea Lorenz has recently reported the occurrence of marked lymphocytosis (76 to 78 per cent.) in the *cerebrospinal fluid.*

CIRRHOSIS OF THE LIVER

(Atrophic form; type, Laennec; chronic diffuse interstitial hepatitis)

Essential Factors.—Secondary anemia; irregular hyperleukocytosis; presence of complement binding substances in the serum; digestive glucosuria; increased ammonia output and acidosis; irregular albuminuria and choluria; ascites.

The Blood.—*Red Cells and Hemoglobin.*—During the early stages of hepatic cirrhosis the blood picture shows no abnormalities whatever, and there can be no doubt that the disease can make very considerable progress even without any manifest influence upon the general health. When this, however, begins to suffer, first, as the result of gastro-intestinal catarrh, diarrhea, and hemorrhages, later, in consequence of the hepatic lesion as such and associated complications, such as chronic nephritis, tubercular peritonitis, etc., anemia develops and may become quite intense. Frequently this is in part obscured by the development of a variable grade of relative polycythemia, referable to a general absolute loss of fluid from the body, or its withdrawal in the form of transudates (ascites, edema). Da Costa places the average loss of hemoglobin at about 40 per cent. and the corresponding loss of red cells at 30 per cent., so that the color index is usually a little less than 1. Of his 40 well-advanced cases, 18 gave a count between 3,000,000 and 4,000,000, 10 one of 2,000,000 to 3,000,000, while in 10 others practically normal figures were obtained. Exceptionally, and usually in marked bleeding cases, the anemia may be more severe, viz., 1,500,000 to 2,000,000. According to Grawitz withdrawal of a large ascitic effusion may cause a drop in the red count, owing to the relief of capillary stasis and the associated polycythemia. Other observers have occasionally noted an increase and referred this to blood concentration in consequence of the rapid subsequent recurrence of the transudate.

The Leukocytes.—In the majority of uncomplicated cases the leukocyte count is normal or diminished; in others there may be a moderate

hyperleukocytosis of the neutrophilic type. In Da Costa's series a count higher than 10,000 was only noted in four. Generally speaking, there is a greater tendency to hyperleukocytosis in the cases associated with jaundice, which, however, is scarcely due to the icterus itself.

The Serum.—In several cases of hepatic cirrhosis I have found that the serum of the patient gives intense complement fixation, in the absence of any added antigen.

The Coagulability.—The coagulability of the blood is delayed in cases associated with icterus.

The Urine.—In the early stages of the disease the urinary picture is normal, but it is noteworthy that at this time already there may be a marked insufficiency in the carbohydrate metabolism, so that digestive glucosuria develops after the administration of 100 to 150 grams of glucose. When the disease is once well established this insufficiency can be demonstrated in a fairly large percentage of cases, but it is not sufficiently constant to serve as an absolute diagnostic factor. In a series of 95 cases of which I could find records a positive result was noted in 42.

Spontaneous glucosuria is not a feature of hepatic cirrhosis. When it occurs it is the expression of an associated diabetic condition. As such it is not rare, and readily explained by the co-development of an interstitial pancreatitis.

The absolute urea content of the urine is practically unaffected, notwithstanding the fact that there has been an extensive loss of parenchyma. Von Noorden mentions a case where the patient eliminated 34.2 grams in twenty-four hours, three weeks before death. The relative quantity, on the other hand, is sometimes diminished (70 to 79 per cent. of the total nitrogen, as compared with a normal value of about 90 per cent.). The ammonia content is frequently increased, though there may be considerable fluctuations. Von Noorden found 8.5 to 12.6 per cent. of the total nitrogen in the form of ammonia, as compared with a normal of 3 to 5 per cent. The highest values (up to 1.4 to 2.5 grams per diem) are found *sub finem vitæ* after the development of cholemic symptoms. The increased ammonia output in hepatic cirrhosis is now viewed as the expression of an acidosis, and as a matter of fact v. Jaksch and others have shown that the amount of volatile fatty acids, besides lactic acid, may be quite considerable. The amount of uric acid remains unaffected. Leucin and tyrosin have been recorded by only one observer (v. Greco). Others mention the frequent occurrence of albumosuria, which, in turn, is denied by still others. Albuminuria proper, together with cylindruria, is common in advanced cases. In the icteric cases bile pigment and bile acids may, of course, be demonstrated. The amount of urine is subject to considerable fluctuations which are largely dependent upon the existent ascites, the occurrence of diarrhea, of hemorrhages, etc.

Ascitic Fluid.—The ascitic fluid in hepatic cirrhosis may be viewed as a typical transudate. The amount is variable, but often surprisingly large. Twenty liters have been removed at one time. After the ascites has once become developed it usually remains a constant symptom to the end. Hemorrhages and active catharsis may cause a temporary diminution in the amount of fluid, but after every drop there is sooner or later a corresponding increase. Cytological examination reveals a predominance of endothelial plaques. As an unusual finding allantoin may be mentioned, which was once demonstrated by Moscatelli.

CIRRHOSIS OF THE LIVER

(Hypertrophic form; type, Hanot; biliary cirrhosis)

Essential Factors.—Marked secondary anemia; relatively frequent hyperleukocytosis of the neutrophilic type; cholemia; presence of complement binding substances in the serum; delayed coagulation; occasional bacteriuria; choluria, albuminuria and cylindruria; frequent absence of ascites.

The Blood.—*The Red Cells and Hemoglobin.*—The tendency to anemia is greater in this form than in the ordinary atrophic variety. Da Costa, in a series of sixteen cases found the loss of red cells about equal to the loss of hemoglobin and occasionally exceeding it, so that an index was obtained which was essentially normal. Hayem found higher indices (1.27 to 1.46), and speaks also of a corresponding tendency to macrocytosis. Cabot mentions a similar case. Da Costa's average count was 2,895,000 and the average hemoglobin value 52.9 per cent., while the corresponding figures in the atrophic cases were 3,526,000 and 60 per cent. respectively. The lowest amount was 1,100,000 and the lowest hemoglobin figure 20 per cent. On rare occasions a marked polycythemia is observed in lieu of an oligocythemia. Two cases of this kind were seen at the Hopkins with 7,800,000 and 8,500,000 red cells respectively.

The Leukocytes.—According to Hanot and Mennier the leukocyte count is increased in all cases of biliary cirrhosis, their figures varying between 9000 and 21,800. Other writers admit that the tendency to hyperleukocytosis is greater in this than in the atrophic form, but they have not found the condition to be constant. In Da Costa's series the count in nine of the sixteen cases was below 10,000. When it exists the hyperleukocytosis is of the neutrophilic type. It is frequently, no doubt, referable to complicating infections. (See Bacteriology.)

The Serum.—As jaundice is one of the cardinal symptoms of the disease the serum is markedly bile-tinged. This is often quite marked at a time already when the visible jaundice is not as yet very mani-

fest and when bile pigment cannot be recognized in the urine by mere inspection.

In two cases of this order (the only two) I could also demonstrate extensive complement fixation by the serum alone, in the absence of any added antigen. Whether or not this could be attributed to antecedent syphilis I am unable to say; a history suggesting this could not be obtained.

Coagulation.—The coagulability of the blood is very much diminished owing to the associated jaundice.

Bacteriology.—In several cases of Hanot's cirrhosis bacteriological examination of the blood has revealed the presence of organisms. Netter mentions the occurrence of staphylococci and Kirikow of diplococci.

The Urine.—The urinary findings in biliary cirrhosis are essentially the same as in the atrophic variety (which see), excepting the more or less constant presence of bile pigment and bile acids in the former variety. To the early development of jaundice, no doubt, the relatively earlier appearance of albumin and casts is referable.

Transudates.—Extensive ascites may develop in the later stages of the disease, but is not one of the cardinal symptoms. Frequently there is no effusion at all or one only of slight degree. The character of the transudates, however, is the same, the only difference being the presence of much bile pigment in the biliary form.

CONJUNCTIVITIS ACUTA

Bacteriology.—While ophthalmia neonatorum is associated with the gonococcus in about one-half of the cases, this organism is much less common in the conjunctivitis of older children and adults. In the non-gonorrheal cases the following may be met with, either by themselves, or variously associated, mixed infections being very common—the Staphylococcus aureus, albus, and citreus, the streptococcus, pneumococcus, Micrococcus catarrhalis, the meningococcus, the Koch-Weeks bacillus, the diphtheria bacillus, Hofmann's pseudodiphtheria bacillus, the xerosis bacillus, Bacillus coli, Morax's diplobacillus, Friedländer's bacillus, the Bacillus pyocyaneus, and the yellow sarcina. Of these, Randolph considers the Staphylococcus albus and the xerosis bacillus as normal inhabitants of the conjunctival sac, but as capable under certain conditions of causing inflammation. Grenouw, in a series of 100 cases, found the Staphylococcus albus in nearly all and Staphylococcus aureus in about 33 per cent., but only in 12 did he feel satisfied, from the large number present and the absence of other organisms, that the staphylococci were etiologically concerned in the inflammatory reaction. As regards their ability to cause sloughing of the cornea Breweston ranks the more important organisms as follows: (1) The streptococcus; (2) the gono-

coccus; (3) Weeks' bacillus; (4) Staphylococcus albus. Regarding the relative importance of Weeks' bacillus the results of the different observers are not in accord. Smith found it only five times in a series of 127 cases, while Pollack claims to have isolated it in 108 cases out of 145; Meyerhof's results coincide with those of Pollack (157 positive cases out of 300).

The diplobacillus of Morax and Axenfeld seems to be relatively unimportant, causing symptoms so slight that the condition scarcely merits being classed as a conjunctivitis.

The pneumococcus cases, according to Hastings, are apparently always mixed infections, and relatively but little virulent.

The meningococcus has been isolated from the conjunctiva of meningitis cases in only a few instances, but there is reason to suppose that it would be found more frequently if systematically sought for. Councilman and his co-workers noted conjunctivitis in 10 cases of epidemic meningitis out of 111, and Davis in 8 out of 31.

CYANOSIS (ENTEROGENOUS)

(Cyanotic polycythemia; Osler's disease; autotoxic enterogenous cyanosis)

Essential Factors.—Absolute polycythemia; increased hemoglobin value; increased specific gravity and viscosity; irregular hyperleukocytosis and variable leukocytic formula; albuminuria and cylindruria.

The Blood.—*The Red Cells and Hemoglobin.*—In all cases of enterogenous cyanosis absolute polycythemia and abnormally high hemoglobin values are constant factors. In the first series of 9 cases collected by Osler the highest count was 12,000,000; in eight it was above 9,000,000, and in the ninth it was 8,250,000. The findings of subsequent observers range within the same limits. Some of the French observers (Vaquez) maintan that, whereas in congenital heart disease and its coincident polyglobulism the diameter of the red cells is increased from 7.5 to 8.5 μ (hyperglobulism), this is not observed in the idiopathic form of polycythemia. Other investigators maintain that there is no essential difference in this respect. It is important to bear in mind that whereas in pernicious anemia there is a general oligochromemia associated with an individual hypercytochromia (increased hemoglobin content of the individual cell), leading to an increased color index, this does not occur in connection with the general pleiochromemia of absolute polyglobulism. As a rule there are no nucleated red cells. Bence, however, mentions a case where isolated megaloblasts and normoblasts were found. The hemoglobin values in Osler's series ranged between 120 and 200.

The Leukocytes.—The leukocytes are usually not increased, though the variations in Osler's series extended from 4000 to 20,000; in the

majority of cases the number did not exceed 10,000. Unfortunately, the differential count has been reported only exceptionally, so that it is impossible to make any definite statements in this respect. Bence gives one count which showed 80 per cent. of neutrophiles, 6.7 per cent. of eosinophiles, 2.3 of mast cells, 1.7 large mononuclears, and 9.5 per cent. of lymphocytes. Ascoli noted 20 per cent. of eosinophiles, but does not seem to have excluded the possible existence of intestinal parasites. Blumenthal has described an atypical case with numerous myelocytes. Normal values were found in two cases, reported by Parkes Weber, while in another instance, described by Hutchison and Miller, the neutrophiles were increased (82 to 83 per cent.), with coincident normal eosinophile values. The causes of these differences are unknown.

General Characteristics.—The specific gravity of the blood is increased (1.067 to 1.083) and in all cases the viscosity far exceeds the normal. Hutchison and Miller found the co-efficient in their case 11.8 times the co-efficient of the viscosity of water. The coagulation time seems to be diminished. The freezing point in one case of Parkes Weber was 53° C.

The Urine.—Very little is known of the urinary condition of these patients beyond the fact that slight albuminuria with hyaline cylindruria is an almost constant symptom.

CYSTIC KIDNEY

The Blood.—In my perusal of the literature I have only found a few cases in which an account of the blood picture was given. Emerson mentions two with red counts of 4,200,000 and 2,800,000, and white counts of 13,500 and 36,000 respectively. Cabot mentions one, a male, aged fifty-five years, who came under observation supposedly suffering from cancer. His red cells numbered 3,664,000 and the leukocytes 4400, of which 72 per cent. were neutrophiles. An analysis of a large number of cases would probably show that there are no material deviations from the normal for a long time. Later in the disease, especially when chronic uremic symptoms develop in association with digestive disturbances, the patients may become markedly cachectic, however, so that the diagnosis of cancer in view of the abdominal tumor and the general deterioration in the health of the patient is frequently made.

The Urine.—The urinary picture is not at all characteristic. In some cases the amount and composition are perfectly normal; in others oliguria has been noted and in still others polyuria with and without albuminuria. The only significant feature seems to be the occasional presence of blood.

Cystic Contents.—The cystic contents show a variable composition not only in different cases, but even in the different cysts in the same

kidney. Sometimes the material is clear and limpid, almost colorless or tinged a lemon yellow; in others it is turbid and mucinous, and in still others tenacious, colloid, and colored a reddish or brownish tint, owing to the admixture of blood in various stages of decomposition. The odor is urinous or ammoniacal and the reaction neutral or alkaline. In addition to albumins (serum albumin and serum globulin) the material contains urea (often in large amount—up to 6 per cent.), uric acid in solution or in crystalline form, and on microscopic examination corresponding crystals, oxalate of lime, leucin-like globules, red corpuscles, leukocytes, fat globules, epithelial cells, and detritus can be made out; occasionally also cholesterin may be seen.

CYSTITIS

Essential Factors.—Irregular hyperleukocytosis of the neutrophilic type; pollakiuria; pyuria; albuminuria; irregular hematuria; bacteriuria; presence of parasites or their ova; concretions.

The Blood.—The blood picture in cystitis depends upon the underlying cause and the extent and severity of the local lesion. In women mild cases of cystitis are frequently seen without any disturbance of the general health and without any deviation from the normal blood picture. When the disease develops secondarily in the course of a general infection there will, of course, be corresponding changes. Tuberculosis of the bladder, persisting after extirpation of a tubercular kidney, is notorious for the frequent lack of systemic disturbance of marked degree; in the majority of cases only a mild grade of chlorotic anemia is noted. In the cystitis associated with malignant disease of the bladder the blood picture is controlled by the latter. So far as the effect of cystitis upon the leukocytes is concerned there can be no doubt that it can give rise to hyperleukocytosis in itself, though this tendency is often obscured by the nature of the underlying malady. It becomes apparent, however, in a disease such as typhoid fever, where, notwithstanding the primary tendency to leukopenia, the number may be, nevertheless, increased when cystitis develops. Of three cases cited by Thayer, one showed a marked increase (18,000). In one case of cystitis, of my own observation, brought on by excessive use of sandal oil, the abdominal pain and neutrophilic hyperleukocytosis (25,000) suggested the existence of appendicitis and led to operation.

The Urine.—Increased frequency of micturition (pollakiuria) is one of the most common symptoms of cystitis and one of the most distressing. Taken by itself, however, it has only limited value in diagnosis, as there are many conditions in which the same may occur in the absence of cystitis (neurasthenia, hysteria, following the administration of drugs, such as copaiba, cubebs, camphor, cantharides, etc., in the course of various fevers, etc.). The amount of urine

passed at one time is often surprisingly small—in severe cases, indeed, only a few drops, while the total quantity may be normal. The reaction in the majority of cases of both acute and chronic cystitis is acid; ammoniacal decomposition is essentially seen in neglected cases. The amount of albumin, even in severe cases, with pyuria of high grade, is surprisingly small, providing, of course that no renal disturbance exists and that little or no blood is present. The examination should be made as soon as possible after the urine is voided and before extensive destruction of pus cells has occurred. According to Rosenfeld the maximum content of albumin, even in the severest cases of cystitis, does not exceed 0.15 per cent., as contrasted with pyelitis, where it is often 0.3 per cent. Brown states that he has repeatedly diagnosticated cases of pyelitis from a lack of disproportion between the amount of albumin and the degree of pyuria, in several of which there was no suspicion that the kidney was affected, the correctness of the diagnosis being subsequently proved by ureteral catheterization and operation.

In order to determine the relative degree to which albuminuria is referable to contained blood, Goldberg counted the red cells and determined the amount of albumin. He found that there is true albuminuria, if the ratio between the degree of the latter (in percentage) and the number of corpuscles per cubic centimeter is more than 1 to 30,000, while if less than 1 to 30,000 the albumin is accounted for by the blood alone (Brown).

Microscopic examination reveals the presence of pus corpuscles in variable number, the height of the sediment on standing being a fair gauge of their quantity. In very mild cases there may be but few more than would be found under normal conditions, but usually they are very numerous. The amount noted in severe cases is exceeded only in those rare conditions in which a neighboring abscess has broken into the urinary passages. The individual corpuscles are usually well preserved in cases presenting an acid urine. When ammoniacal decomposition has set in, however, it may happen, owing to the disintegrating action of the ammonium carbonate upon the pus corpuscles, that these are no longer demonstrable with the microscope, and that a gelatinous mucoid sediment appears instead, which may escape from the vessel *en masse*, when the urine is poured out (Donné's pus test is based upon this principle).

Blood may be present in macroscopic amount, or it may be demonstrable only with the microscope. Frequently it is difficult to determine the origin of the blood. In cystitis the cells present a normal appearance, unless ammoniacal decomposition has set in, when blood shadows are seen in large numbers. Otherwise loss of coloring matter and extensive crenation suggest that the bleeding has been renal. In cystitis, moreover, the blood is less intimately mixed with the urine than in renal hematuria, so that the corpuscles rapidly settle

to the bottom after the urine has been voided. Blood clots of irregular form and considerable dimensions can only be of vesical origin. Relatively free bleeding, in connection with cystitis, is essentially seen in cases of stone, malignant growths involving the bladder, in tubercular ulceration, and in certain parasitic diseases involving the bladder.

Epithelial cells are always found in cystitis; they are large, flat cells and usually occur singly, while the cells from the pelvis of the kidney are frequently found in groups and dovetailed the one into the other; their number is variable; sometimes, even in acute cases, there are only a few, while in others they are very numerous. If the urine is acid the sediment may contain crystals of oxalate of lime or uric acid; more frequently there are no crystalline elements. If ammoniacal decomposition has set in there will be triple phosphates and ammonium urate; more rarely calcium carbonate is found. In an alkaline urine, the alkalinity of which is due to fixed alkali, crystals of the alkaline phosphates of calcium, together with triple phosphates, may be encountered.

Bacteriology.—Most important in every case is the bacteriological study of the urine. In rare instances, where the cystitis has been produced by the ingestion of chemical irritants, such as copaiba, cubebs, sandal oil, cantharides, anilin, toluidin, naphthalin, etc., the cultures are sterile. Otherwise some organism is found in practically every case, even though the primary disease of the bladder has been of non-bacterial origin, secondary infection readily taking place in the diseased organ. The flora of the bladder is quite diverse. In those cases in which the cystitis develops secondarily in the course of a systemic bacterial invasion the corresponding organism is usually met with, such as the typhoid bacillus in typhoid fever, streptococci in erysipelas and scarlatina, the pneumococcus in pneumonia, etc.

Of special interest are those cases which develop after operation. In a very careful study of 26 cases of this kind (25 women and 1 man), Brown found the colon bacillus in 15, Staphylococcus albus in 5, aureus in 2, Bacillus pyocyaneus, Bacillus typhosus, and Proteus vulgaris each in one (the latter in the male patient). In a further study of 24 chronic cases, Brown found the colon bacillus 12 times, Staphylococcus aureus in 3, the albus in 2, and a non- or slowly liquefying urea decomposing white staphylococcus in 2, while in 2 cases the urine was sterile. In 6 cases of tubercular cystitis, 2 of which were associated with pyelitis or pyelonephritis, the tubercle bacillus was demonstrated in all, and in all but one the cultures were sterile; in this one case the colon bacillus grew out though only a suprapubic cystostomy had been performed. The search for the tubercle bacillus in these cases is often very tedious; usually the organisms are present in small numbers and are found only after a protracted search; more rarely they are abundant.

The length of time that bacteria may persist in the urine after they have once gained access to the bladder is quite variable. When the infection has taken place in the course of an acute disease they may, and usually do, disappear as convalescence becomes established, but at times they remain for months and years. Remarkable observations of this order have been made in typhoid fever. Young gives the history of a patient in whom cystitis developed during an attack of typhoid fever. The organism could still be demonstrated in the urine after several years. An added infection with the pneumococcus subsequently occurred, and four months later typhoid bacilli and pneumococci both were present in considerable numbers. Cystoscopic examination showed a chronic ulcerative cystitis. Since then a number of other cases have been reported in which the typhoid bacillus could be demonstrated in the urine years after the original infection. Much more common are the persistent colon bacillus infections with associated cystitis, which are notably seen in women. There are many cases of this order in which the condition has persisted for five, ten, and even twenty years. The majority of these cases, indeed, give a history of cystitis dating back a number of years, when they first came under observation, and in many of them the condition persists in spite of treatment, with remissions and exacerbations for years thereafter. Brown cites a couple of cases in which cystitis had manifestly existed for several years, but in which the offending microörganisms had died out.

Wright has suggested that much good might be accomplished in these chronic cases by treatment with homologous vaccines. My experience in this direction has been rather meager, but so far as it goes the results have been disappointing. I have not been able to bring about the disappearance of the colon bacillus in a single instance in this manner.

In those cases of cystitis which are associated with and probably referable to infections with Distomum hematobium (Bilharzia hematobia) the corresponding ova are found in the urine, where they may be present in large numbers. Microscopic examination usually also shows the presence of red cells in large numbers. Sometimes the entire bulk of the urine is blood-tinged, but more often only the last few drops contain blood; in this portion the eggs of the parasite are most likely to be found. The condition is exceedingly common in Egypt, where the greater portion of the Fellah and Coptic population is infected. It is likewise of frequent occurrence in South Africa and has also been observed in Mesopotamia and apparently in Arabia. In the United States a few cases have been observed which were undoubtedly imported; the same holds good for Europe.

In rare cases echinococcus hooklets and fragments of cyst wall may be found. Bothriocephalus liguloides has been found in a few cases in Japan and China, and it appears that in one or two instances

Eustrongylus gigas has been observed, though little is mentioned regarding the condition of the bladder.

Infusoria, notably the trichomonas vaginalis, are relatively common in cases of cystitis, though very few observers have made any note of the frequency of their occurrence. I have notes of several cases of this order, but can unfortunately not state what the bacteriological findings were at the time of examination, nor have I records of cystoscopic examinations. Pus was present in all and blood in several. The number of the organisms was variable, but usually large.

Other writers mention the occurrence of amebæ in the urine in connection with cystitis. I have no personal notes of such findings.

Tumor particles associated with cystitis have at times been observed in corresponding cases. Such occurrences are regarded as exceptional, but I have no doubt that they would be more frequently found if carefully sought for.

In cases of calculous cystitis concretions may at times be observed; such findings were probably more common in former years than at present, where the diagnosis of stone is made so much earlier and appropriate treatment is more promptly instituted. Renal calculi now are much more common than vesical concretions, and no doubt for the reasons just stated.

CYSTONEPHROSIS

(Hydronephrosis; pyonephrosis)

Essential Factors.—Irregular neutrophilic hyperleukocytosis; explosive polyuria.

The Blood.—The blood picture in cystonephrosis depends to a great extent upon the nature of the underlying cause. When the condition develops as the result of the impaction of a calculus or from pressure of a non-malignant tumor, or in consequence of torsion of a ureter in case of floating kidney, etc., *i. e.*, from causes which in themselves are not necessarily associated with anemia, a normal blood picture, so far as the red cells and hemoglobin are concerned, will be found. If, on the other hand, a malignant growth (uterus, prostate) or some active pelvic inflammatory condition causes the urinary stasis, the blood picture may be considerably altered. Unfortunately there are no data available from which it would be possible to judge the effect of the cystonephrosis upon the leukocytic formula by itself. Cabot mentions four cases of hydronephrosis with leukocyte counts varying between 6500 and 28,600, and two cases of pyonephrosis, in one of which 16,200 leukocytes were counted with 85 per cent. of neutrophiles. In the other the first count was 9000; about a month later it was only 6650; three days later, at operation, a pint of fetid pus was removed, after which the patient died.

The Urine.—The amount of urine which is secreted in cystonephrosis is quite variable, and depends to a great extent upon the degree to which the urine from the affected side is blocked. When double hydronephrosis exists, which is sometimes observed congenitally, there is, of course, complete anuria; the same may occur if for any reason, in unilateral disease, the other kidney ceases to function. In both cases, of course, uremia will of necessity develop if the condition is not relieved. When the one side functions normally, while the other is blocked entirely, a normal quantity may be eliminated. This will also occur when the cystonephrosis is partial, while the remaining portion of the kidney is not blocked. In both cases polyuria may also exist continuously. In intermittent cases, where the sac empties itself from time to time, there will be corresponding periods of exaggerated urinary flow, which are followed in turn by relative oliguria or normal elimination. These changes, when associated with corresponding changes in the size of the renal tumor, are very characteristic.

The chemical and microscopic composition of the urine is, of course, exceedingly variable, and depends upon the question whether the integrity of the kidney, and especially its pelvis, is involved, and whether or not the urine which is voided is derived only from the normal or from the affected side as well. When this is blocked entirely and the other kidney is normal, a normal urine will be found. The same will be observed in cases of hydronephrosis in which there is no inflammatory disease involving the kidney, even though the obstruction be only partial, while the urinary picture will be essentially the same as that noted in pyelitis from whatever cause, when this is the essential lesion, providing, of course, that the contents of the sac have found their way into the bladder. In that event there may be pus, renal or pelvic epithelium, blood corpuscles, tube casts, and various crystalline elements, while the bacteriological findings will depend upon the character of the pyelitis.

As in pyelitis, ureteral catheterization should be practised in all doubtful cases, or whenever operative interference is intended.

DEMENTIA PRÆCOX

In schizophrenia Itten found the red cells much increased, especially in catatonic cases and during stupor. In acute cases, and chronic hebephrenics, the neutrophilic leukocytes were slightly increased, while with the subsidence of the acute symptoms there was a tendency to lymphocytosis. This is especially marked in unfavorable cases, while in cases showing improvement the neutrophiles as well as the eosinophiles increased.

According to the researches of Fauser, the serum of dementia præcox cases contains a ferment which is capable of splitting sex gland tissue, the serum of males reacting with testicular proteins

and that of females with ovarian and tubular material. In the majority of cases a reaction was also obtained with brain cortex, and now and then with thyroid gland. These results have been confirmed by Fischer, Wegener, and my own investigations, which were made in association with Judd and Ensor. They are especially significant in view of the negative findings which appear to be the rule in manic-depressive insanity, hysteria, and other "purely functional" psychoneuroses.

DENGUE

Essential Factors.—Leukopenia with lymphocytosis.

The Blood.—*The Red Cells.*—According to Carpenter, Sutten, Vedder and others, there is no diminution of the red cells in dengue.

The Leukocytes.—In some cases there is a normal absolute count, but in the majority there is a striking leukopenia; hyperleukocytosis has not been observed. Regarding the differential findings, I append the following data, taken from Vedder: A marked reduction in the polymorphonuclear (neutrophilic) count takes place early in the disease; it is very evident by the second or third day, at latest, and this reduction is constant with slight variations throughout the course of the disease, until convalescence is complete, and possibly lasts for some time after convalescence. Coincident with this decrease in polymorphonuclears there is an increase in the small lymphocytes, which takes place with equal rapidity and is of similar duration. There is a gradual though much more moderate increase of the large "lymphocytes" (these no doubt are all large mononuclears and not lymphocytes in the original sense of the term). There is a similar gradual, but slight increase of the eosinophiles, which at first tend to subnormal values. The predominance of cells is transferred as early as the second day in fully one-half of the cases, and in practically all cases by the third day, from the neutrophiles to the lymphocytes.

Vedder suggests that it should be possible to distinguish dengue from yellow fever by the differential count (see the latter disease).

Parasitology.—Graham has described a protozoan parasite in the red cells of dengue patients, but his work has not been confirmed.

The Urine.—The urinary picture of dengue has not received any detailed study.

DIABETES

Essential Factors.—Irregular polycythemia; Bremer's reaction; hyperleukocytosis in connection with coma and complicating infections; decrease in the alkalinity of the blood during coma; increased sugar content; Williamson's reaction; increased fat content in severe cases; polyuria; glucosuria; acidosis (increased ammonia content, acetonuria, diaceturia, and β-oxybutyric acid elimination).

The Blood.—*The Red Cells and Hemoglobin.*—In the majority of cases of diabetes the number of red cells and the amount of hemoglobin is about normal at the time when the patient first presents himself for examination. This may remain so throughout the course of the disease. Not infrequently, however, the findings become variable as the disease progresses, periods of moderate oligocythemia alternating with periods of moderate polycythemia, and these again with normal findings. The cause of these irregularities is not very clear; usually they are referred to variations in the concentration of the blood owing to irregularities in the elimination of water. Sometimes the polycythemia is unquestionably only relative, as is evidenced by the emaciated condition of the patient and the manifest anemia. But in others such a discrepancy is not apparent, and it has hence been argued that the polycythemia may at times at least be real. High values are commonly seen during coma. Grawitz mentions a case in which the red cells rose to 6,400,000 five hours after the onset of the coma, whereas three weeks before the number had been 4,900,000.

Considering the number of the red cells, the hemoglobin is often considerably reduced. In James' series, in which the corpuscles ranged between 3,550,000 and 6,730,000, the lowest and highest hemoglobin values were 52 and 112 per cent. In the Hopkins series mentioned by Emerson the count was below 4,000,000 in 3 cases (the lowest being 2,000,000), between 4,000,000 and 5,000,000 in 13, between 5,000,000 and 6,000,000 in 19, and above 6,000,000 in 4; 3 other cases were at times over 6,000,000.

The morphological study of the red cells reveals no abnormalities. Bremer, however, has pointed out that a difference exists in the affinity of diabetic blood for certain anilin dyes, as compared with non-diabetic blood. For whereas the latter is readily stained with Congo red, methylene blue, eosin, and others, diabetic blood is more or less refractory, while certain dyes, like Biebrich scarlet, which readily stain diabetic blood, do not color non-diabetic specimens. Upon this principle Bremer's *diabetic blood test* is based. (See Technique.) His claim that the reaction is pathognomonic of diabetes (sc. glucosuria) and may even yield positive results in the prediabetic stage of the disease, and when the sugar has temporarily disappeared from the urine, has been confirmed in all essential points. A few interesting exceptions, however, have been noted. In Bremer's series of diabetic cases a negative result was obtained but once, and in this instance he believes that the diabetes was of the renal type and analogous to the phloridzin diabetes of animals in which he similarly obtained negative results, while phloroglucin diabetes produced a positive reaction. Lépine and Lyonnet have reported a positive result in a case of leukemia, which Bremer, however, refers to faulty technique. In Basedow's disease, very curiously, a similar

reaction was obtained by Eichner and Folkel, as also by Badger. Hartwig found it also in multiple neuritis and Hodgkin's disease. My own experience with the reaction has been quite limited, but has borne out Bremer's claim; the technique, however, is difficult, and for this reason, no doubt, the reaction has never won popular favor.

The Leukocytes.—In uncomplicated cases of diabetes the leukocyte count is normal. During coma, however, there is sometimes marked hyperleukocytosis (12,500 to 49,000). Otherwise the occurrence of hyperleukocytosis indicates the existence of complications, such as furunculosis, gangrene, septicemia, etc. In gangrenous cases the counts may vary between 8200 and 20,000. The differential count in uncomplicated cases quite frequently reveals the existence of a lymphocytosis and occasionally of a slight eosinophilia; in cachectic cases a few myelocytes may be encountered.

The glycogen reaction often shows an increased amount of extra- as well as intracellular iodophilic material.

The Specific Gravity.—The specific gravity of the blood varies between 1.054 and 1.060.

The Alkalinity.—The alkalinity remains unaffected, so long as β-oxybutyric acid and other fatty acids do not appear in the urine. When this occurs, however, it diminishes, the lowest values being found during coma. In 3 cases of this kind Magnus-Levy's initial values (normal = 320 mgrms. NaOH for 100 c.c. of blood) were 361, 298, and 324, and the final figures 144, 124, and 154. There is thus a very manifest acidosis. *Sub finem vitæ* the reaction of the blood may actually be acid. Corresponding to this condition there is during coma marked diminution in the alkali tension of the blood.

The Sugar Content of the Blood.—Early in the disease the sugar content of the blood is probably always normal, *i. e.*, lower than 0.08 to 0.09 per cent., even though glucosuria exists. Later, however, it rises, and in well-established cases high figures are the rule; 0.3 to 0.4 per cent. are common values. More rarely one finds 0.78 and even 10 per cent. Very curiously the blood sugar content is apt to be higher than normal, even at times when the urine has been rendered sugar-free in consequence of a rigid diet.

The only exception to the rule that diabetes is associated with hyperglucemia is noted in the so-called renal type of the disease which has been described by Klemperer, and which is analogous to the phloridzin diabetes that can be produced artificially in animals.

Williamson's diabetic blood test (see Technique) is based upon the existence of hyperglucemia in diabetes and its absence in other diseases, as well as in non-diabetic glucosuria. The reaction is said to be demonstrable in diabetics even at a time when the urine has been rendered sugar-free by appropriate diet.

The Fat Content of the Blood.—According to the most recent studies of the problem, the fat content is not materially increased beyond the

maximal normal limit (about 1 per cent.), excepting in very severe cases and at a time when acidosis of high grade exists. All cases of extensive acidosis, however, are not necessarily associated with lipemia. In marked cases of lipemia the serum is distinctly milky in appearance, and it is interesting to watch this vary with the condition of the patient. It may thus be observed that with the onset of coma the serum becomes quite turbid and clears up again as the coma disappears. The actual amount of fat usually does not exceed 4 to 6 per cent., but in isolated cases still larger amounts have been found (viz., 15 to 18 per cent. ethereal extract, of which 2.6 per cent. was referable to cholesterin, in one case). In such cases fat emboli may be found post mortem plugging the vessels of various organs, such as the brain, the lungs, and the kidneys.

The Urine.—Generally speaking, there is a direct ratio between the sugar content of the urine, the *specific gravity*, and the *amount*, as expressed in the following figures (v. Noorden):

Amount of urine.	Specific gravity.	Percentage of sugar.
1500 to 2500 c.c.	1025 to 1030	2 to 3
2500 to 4000 c.c.	1030 to 1036	3 to 5
4000 to 6000 c.c.	1032 to 1040	4 to 7
6000 to 10,000 c.c.	1036 to 1046	6 to 9

To this rule, however, there are many exceptions, and in older patients particularly it is not uncommon to meet with an elimination of 3 to 4 per cent. of sugar with a normal output of urine. 5000 to 6000 c.c. may be regarded as common values when the patient is on an unrestricted diet, and exceptionally the quantity may reach 10 liters or more; when the sugar is reduced by appropriate diet the flow of urine undergoes a corresponding reduction. The highest figure recorded is 28 liters, and the highest specific gravity 1.074. In contradistinction to the polyuria of chronic interstitial nephritis, the greater amount of urine in diabetes is secreted in the day time.

Recent researches have demonstrated that the inability of the diabetic to oxidize what may be termed a normal amount of carbohydrates is not of necessity limited to glucose, but may extend to various other sugars as well, as will be shown below. While this insufficiency toward other sugars is inconstant, however, glucosuria is observed in all cases of diabetes and is one of the essential factors of the disease. Its degree is exceedingly variable, and it has been the custom clinically to divide diabetics into three classes according to the amount of sugar which is eliminated. In a general way this is admissible, but it must be borne in mind that this classification has only a limited reference to prognosis, since a patient placed in the first class owing to the mild degree of his glucosuria may in a short while develop serious symptoms, while apparently severe cases may assume the more benign type.

In the first group the urine only contains sugar when the diet contains carbohydrates (amylacea). Here subdivisions are possible in accordance with the extent to which the patient is capable of oxidizing varying amounts of starchy food. In some of these cases glucosuria is only produced if all starchy food is withdrawn, while others can consume 50, 100, and 150 grams without responding with glucosuria. This group comprises the mild cases, and in these the daily output of sugar usually does not exceed 50 grams while the patient is on his usual diet. In the second group, comprising the moderately severe cases, the glucosuria can only be caused to disappear if in addition to the complete withdrawal of starchy food the patient's albumins are restricted such that the daily output of total nitrogen amounts to from 10 to 18 grams. On an unrestricted diet such cases eliminate from 100 to 250 grams. The third group finally includes the severe cases, in which the glucosuria can only be reduced if, besides the withdrawal of starches and sugar, the nitrogenous output is diminished to less than 10 grams in the twenty-four hours. The daily elimination in such cases may approach 500 grams or more. In isolated cases 1000 grams and even 1500 have been noted. *Sub finem vitæ* the elimination of sugar may diminish to mere traces, or it may cease altogether.

As I have said before, not too much reliance should be placed upon this scheme, as considerable variations to and fro may occur in any diabetic. Such changes may occur spontaneously or in consequence of changes in diet. Practically important is the fact that periods of strict dieting may increase the tolerance for carbohydrates for a certain length of time. In light cases this may be quite considerable.

While glucose and the corresponding polysaccharids increase the glucosuria, lactose and levulose do so to a less extent. Oatmeal and potato starch are especially well borne. An increase of the proteins produces a slight increase of the sugar, while fat is without effect. Active muscular exercise causes a decrease and mental emotions an increase.

Intercurrent diseases may diminish or increase the glucosuria. The first is notably seen in nephritis. In acute infections the results are variable; sometimes there is an increase, at others a decrease.

From the standpoint of diagnosis it should be remembered that the glucosuria of diabetes in contradistinction to other forms, and uninfluenced by diet, is continuous.

Acidosis.—In all cases of diabetes there is a tendency to acidosis, which finds its expression in the urinary picture in an increased elimination of *ammonia* and in the appearance of the *acetone bodies*, viz., acetone, diacetic acid, and β-oxybutyric acid. In the light cases this tendency unquestionably also exists, but may not be manifest. It may, indeed, be questioned whether any case in which the existence of acidosis can be demonstrated can be viewed as a light

case. In the severer cases, on the other hand, more or less extensive acidosis is the rule, and during coma the condition is at its height.

In the milder cases of acidosis acetone only is found; the presence of diacetic acid, as well, indicates a firmly established condition, and in the most serious cases β-oxybutyric acid can be demonstrated together with the two others. According to Herter diabetic coma may be preceded by days, weeks, or months, during which there is a large excretion of oxybutyric acid (20 grams or more in the twenty-four hours). The persistent excretion of more than 25 grams indicates impending coma. Much larger amounts, however, may be met with, such as 50 to 60 grams or more. The amount of acetone may exceed 5 grams per diem. When it reaches 0.4 to 0.5 gram, diacetic acid is usually also present and can be demonstrated with the chloride of iron test (which see). When oxybutyric acid appears, the acetone content is usually above 0.8 to 1 gram. In advanced cases the oxybutyric acid increases to such an extent that its amount constitutes two-thirds to three-fourths of the total amount of acetone bodies; reports of still larger quantities are based upon faulty technique.

Marked acidosis develops when diabetic patients are placed on starvation, but disappears after they have become sugar free.

Unfortunately we have no ready method by which we can quantitatively follow the development and course of acidosis by a determination of the acetone bodies themselves. The same purpose, however, may be accomplished by the estimation of the ammonia content of the urine, which can be readily done with very small laboratory facilities. The ammonia curve follows the acid curve very closely, and is, indeed, determined by the latter, as the body tries to protect itself against the acid intoxication by allowing a portion of its nitrogen to leave in this form instead of being transformed into urea.

In mild cases the daily elimination of ammonia varies between 1 and 1.5 grams, corresponding to 10 to 12 per cent. of the total nitrogen. In severe cases 4 to 6 grams are average quantities, which may be observed for weeks and months (20 to 25 per cent. of the total nitrogen). Still larger amounts, such as 10 grams and more, are exceptional even in the stage preceding coma. In one instance v. Noorden noted 10.5, corresponding to 45 per cent. of the total nitrogen. According to Magnus-Levy, every gram beyond the amount which would correspond to the extent of the albuminous diet represents 6.12 grams of oxybutyric acid. The administration of fixed alkalies will, of course, bring about a corresponding decrease in the amount of ammonia.

In the absence of facilities for the estimation of ammonia the chloride of iron test for diacetic acid may be used in demonstrating and following the course of the acidosis.

As has already been pointed out, marked lipemia, which can be recognized with the naked eye in a centrifugalized specimen of blood, is commonly observed when acidosis is marked.

Nitrogen Partition.—In the absence of acidosis the *urea-nitrogen* fraction is practically normal, as is also the ammonia fraction, average figures being 80 per cent. and 5 to 6 per cent. respectively. This remains the same on a diet rich in proteins, even though the absolute ammonia content is increased. When the percentage amount of the latter undergoes a material increase, as the result of acidosis (10 to 45 per cent. of the total nitrogen), there is, of course, a corresponding decrease of the urea fraction. If the patient is on a diet rich in proteins the actual amount of urea may be higher than what would correspond to a diet of average composition, while the relative amount may in reality be diminished. A high urea content as such can hence not be viewed as excluding an increase in the ammonia values, and the urea estimation in diabetes can hence never take the place of the ammonia estimation.

Uric Acid.—The uric acid content of the urine is not increased in milder cases of diabetes (0.35 to 0.45 gram of endogenous origin), while in severe cases there is at times an increased (toxic) destruction of nuclear substances (0.75 to 0.95 endogenous) without a corresponding breaking down of proteins as such.

Kreatinin.—The kreatinin is increased in proportion to the increased consumption of animal food (?) and the destruction of the patient's own tissues.

Oxalic Acid.—The elimination of oxalic acid is independent of the diabetic process and largely regulated by the character of the diet. Occasional higher values than normal must be due to complicating conditions.

Glucuronic Acid.—While the formation of glucuronic acid has been generally regarded as serving the purpose of binding various aromatic substances which have been introduced from without, or have originated in the body itself, and that glucuronic acid in the free state does not occur in the urine, P. Mayer has pointed out that glucuronic acid may, after all, occur uncombined, and that such an event indicates a deficient oxidation of sugar and may be viewed as a possible precursor of diabetes. If this were proved it would manifestly be an important matter to establish the existence of such a condition, but reports thus far do not support Mayer's contentions.

Presence in the Urine of Diabetics of Sugar Other Than Glucose.—*Levulose.*—This is only rarely found in the urine of mild cases of diabetes, while in severe cases it is commonly present and may amount to 0.3 to 1.2 per cent., representing as much as $\frac{1}{5}$ or $\frac{1}{4}$ of the total quantity of sugar. This type of levulosuria is spontaneous, as it occurs irrespective of the administration of levulose. In addition to it there is a digestive form which also occurs in the

diabetic patient. Generally speaking, levulosuria may be regarded as a more extensive metabolic abnormality than glucosuria.

Cane Sugar.—Cane sugar has never been found in diabetic urine.

Maltose.—Maltose together with glucose was first found in the urine of a patient supposedly the subject of pancreatic diabetes, associated with an acholic condition of the stools. Since then it has been observed in several other cases of diabetes, but it is, after all, a rare occurrence. In one case the amount was 27.8 grams pro liter.

Spontaneous *lactosuria* does not occur in diabetes excepting during the puerperal period, when the same may be noted in non-diabetic individuals. At other times the diabetic responds with glucosuria to the introduction of lactose, though there may be a considerable degree of tolerance in some cases.

Dextrin.—Dextrin, which occurs in normal urines in small amount (about 0.15 gram per diem), is commonly found to be increased in diabetes (usually between 0.8 and 24.4 grams). The amount is generally proportionate to the severity of the case.

Pentose.—Pentose (i-arabinose) is frequently found in the urine of diabetic patients, particularly in those of a severe type. As a substitute for sugar it is inapplicable.

Albuminuria and Cylindruria.—Albuminuria is observed in from 25 to 35 per cent. of all diabetics, and may occur irrespective of the gravity of the case. In the majority the condition is merely "functional," and frequently will disappear, like other complications, such as furunculosis, pruritus, neuritis, amblyopia, etc., if the glucosuria can be caused to disappear and the general condition of the patient is improved by appropriate adjustment of the diet. In other cases it continues for years. Usually the amount is small, but it is subject to considerable fluctuations. Actual nephritis (parenchymatous as well as interstitial) is observed in a relatively small percentage of the cases, and, as has been pointed out already, the glucosuria is then apt to diminish.

In cases of marked acidosis, particularly in association with coma and frequently preceding this by a short time, large numbers of hyaline and granular casts appear in the urine, irrespective of the grade of albuminuria—the so-called coma cylinders of Ebstein and Külz. In cases which recover from the coma the casts may disappear with the next attack.

Pneumaturia.—In rare cases, owing to accidental infection either with yeast cells or butyric acid producers, fermentation of the sugar may occur already in the patient's bladder. The gases depend upon the nature of the fermentative process. Müller found H, N, CO_2, and traces of CH_4; the nitrogen has probably diffused into the bladder from the blood. Senator and others obtained CO_2.

The Saliva.—The reaction of the saliva is frequently acid, which is apparently not referable to fermentative processes in the mouth.

The sulphocyanide reaction may be absent. In acidosis cases acetone can be demonstrated.

The Gastric Juice.—The gastric juice shows no abnormalities of moment. Sometimes normal values are met with; then again, hyperchlorhydria, and at still other times, anachlorhydria.

DIPHTHERIA

Essential Factors.—Hyperleukocytosis; septic factor; presence of the diphtheria bacillus in the exudate; tendency to albuminuria.

The Blood.—*The Red Cells.*—The red cells, owing to concentration of the blood, no doubt, are usually found increased or at least at the maximal normal limit during the first week of the disease; 5,100,000 to 5,600,000 may be regarded as average values. In the second and third weeks some cases still show these high or even higher values (6,800,000), but in others a loss of about 500,000 cells is met with; this may even amount to 2,000,000. The tendency to loss of the red cells is apparently more marked and more frequent in those cases which have received no antitoxin. Cabot gives a series of 23 cases treated with antitoxin, only 3 of which showed any considerable diminution in the number of the red cells, and in these the loss was less than 400,000. Where anemia does occur the return to normal values is rather slow. Nucleated red cells (normoblasts) are occasionally seen.

The Hemoglobin.—This is reduced about 10 per cent. in patients who receive no antitoxin; greater losses occur in those cases in which a more marked reduction of red cells has taken place. During convalescence the gain in coloring matter occurs more slowly than that of the red cells.

The Leukocytes.—Barring both the unusually mild and the unusually severe cases, hyperleukocytosis is the rule (in fully 90 per cent. of all cases). The increase is usually progressive during the first few days; then it subsides; but in some instances higher values than the normal may continue into convalescence. In fatal cases there may be a further rise or a drop. Ewing mentions two cases in which there was no leukocytosis up to the fourth and sixth days respectively. He suggests that in one of these the delayed rise may have been due to a prolonged toxic hypoleukocytosis, as the patient died subsequently. Generally speaking the hyperleukocytosis is proportionate to the amount of exudate present; more specifically, however, it indicates the reactive power on the part of the individual. In severe cases counts of 25,000 to 30,000 are common, and at times higher values are obtained. Ewing reports a case with a count of 72,000, and Felsenthal mentions one associated with a hemorrhagic eruption in which the leukocytes numbered 148,000. Ewing very properly regards this as probably an agonal hyperleukocytosis.

The hyperleukocytosis in most cases of diphtheria is referable to an increase of the neutrophilic elements; on an average they represent 80 per cent. of all the leukocytes present, and of these, the greater number by far are polymorphonuclears; the polynuclears proper (in the sense of Arneth) are much diminished. The eosinophiles also are diminished and may be absent (septic factor); their return is a favorable sign. In a few instances the lymphocytes have been reported as increased at the height of the disease. Ewing thus cites two instances in which the relative counts were 60 and 62 per cent., corresponding to total counts of 72,000 and 22,500 respectively. How frequently lymphocytosis occurs in diphtheria has not been ascertained. During convalescence it is common, as in other diseases, in which the septic factor occurs during the height of the disease. It has, however, also been reported in fatal cases associated with leukopenia.

A small number of neutrophilic myelocytes (metamyelocytes) is a common occurrence in diphtheria and of no particular importance. Larger numbers, viz., from 2 to 14.6 per cent., are regarded as an unfavorable symptom; their absence, on the other hand, cannot be interpreted as a favorable sign, since many fatal cases are seen in which myelocytes cannot be demonstrated.

Degenerative changes in the leukocytes have been described especially by Ewing, Gabritschewsky, and Filé. Many of the cells show a deficiency in chromatin and in the number of the neutrophilic granules. Leukocytic shadows, according to Klein, are seen in all severe cases, and are sometimes very numerous in the fatal septic types. Iodophilia is commonly present.

The use of antitoxin causes a marked drop in the number of the leukocytes. This occurs early, viz., within a half-hour after the injection, and in favorable cases the number does not attain the same height again which was seen in the primary count. The loss in cells may amount to from 4000 to 20,000 per c.mm. No drop is seen in the severe forms of the disease, or if it does occur there is a subsequent rise which goes beyond the former height. In some of the malignant cases the injection of antitoxin is followed by marked leukopenia and death.

The Blood Platelets.—According to Tschistowitch there is a marked reduction in the number of the blood platelets, which continues even after the throat has cleared up entirely.

Bacteriological Examination of the Blood.—The diphtheria bacillus is rarely found in the blood; when it does occur this is almost always *sub finem vitæ.*

The Exudate.—The diphtheria bacillus can be demonstrated in the exudate, either directly or by culture, in all cases. The number in smears is quite variable. In many cases a typical bacillary picture is seen, while in others cocci predominate and careful search is

necessary to find organisms presenting the characteristic morphology. In many of the fatal cases streptococci stand in the foreground. Besides bacteria, smears from the exudate will contain pus corpuscles and epithelial cells in various stages of degeneration, granular detritus, and threads of fibrin. It is of great practical importance to recognize the fact that the organism may remain in virulent form in the upper air passages of individuals who have passed through an attack of diphtheria, for weeks and months after the exudate has disappeared and that such persons are a menace to those with whom they come in contact. The same holds good for attendants upon diphtheria patients. Quarantine restrictions upon diphtheria houses should accordingly not be withdrawn until a bacteriological examination of the throat *and* nose of every inmate shows that all danger of infection has passed.

The Urine.—The general characteristics of the urine in diphtheria are those which are seen in any febrile case. *Albuminuria* is found in fully one-half of the cases, but its frequency seems to vary in different epidemics. As compared with scarlatina, the albuminuria of diphtheria occurs much earlier; it may, in fact, be observed at the very beginning of the disease. Usually the amount of albumin is small, but in severe cases it may be quite large. It disappears with convalescence in the majority of cases, but in some it may be the starting point of more or less permanent renal disturbance. A moderate number of casts is indicative of a more intense irritation. *Hemoglobinuria* (see hematinuria) is occasionally observed, but rather uncommon. *Glucosuria* occurs in some of the cases, but is transitory. Binet obtained a positive result in 29 cases out of 70—27 times in severe infections out of 38, and twice in mild cases out of 32. I have found sugar in 4 out of 32 cases, the infection being of moderate intensity. Hibbard and Morrisey arrived at similar results.

Bookmann claims that the *benzaldehyde reaction* is found in all cases.

The diazo reaction, according to Rivier and others, is uncommon. Of 118 cases examined by Rivier and 44 others collected from the literature, only 10 gave a positive result, and of these 4 should be eliminated, as they occurred in complicated cases; the reaction is thus absent in about 97 per cent. of the cases.

ECLAMPSIA

(See Pregnancy and the Puerperal State)

EMPHYSEMA

Essential Factors.—Irregular anemia with paroxysmal relative polycythemia; irregular hyperleukocytosis with hypereosinophilia; mucoid expectoration.

The Blood.—*The Red Cells and Hemoglobin.*—So long as no cyanosis exists anemia of a mildly chlorotic type is observed in many cases; this may be referable to the impaired state of the patient's nutrition, to a complicating nephritis, cirrhosis of the liver, tuberculosis, etc. In other cases, where the general health is fair, the number of red cells and hemoglobin remain normal. In cyanotic cases, on the other hand, there is frequently a capillary polycythemia with correspondingly high hemoglobin values, which may become further increased if asthmatic attacks supervene.

The Leukocytes.—When the condition is quiescent the number of the leukocytes is normal. During exacerbations of the associated bronchitis or in connection with asthmatic attacks, however, hyperleukocytosis is frequently observed. The leukocytic formula will depend in a measure upon the nature of the bronchitis. Most characteristic condition is an increase of the eosinophiles which is seen in almost all cases, no matter whether hyperleukocytosis exists or not. This is usually moderate, not exceeding 10 to 15 per cent., but exceptionally much higher values are observed. Temporarily the eosinophilia may disappear or diminish, when infections supervene which in themselves give rise to the septic factor (pneumococcus, Micrococcus catarrhalis). (See also Asthma.)

The Sputum.—In some cases of emphysema there is no sputum, or only a small amount of bluish-white mucoid material is expectorated in the morning. When the chronic bronchitis is marked or when acute exacerbations occur it becomes more abundant and may at times be quite copious (up to a pint a day). The mucoid character predominates. Purulent material is usually expectorated only in small amount. Occasionally the sputum is streaked with blood. In rare instances casts of some of the bronchioles are found. The morphological elements are for the most part neutrophilic leukocytes and alveolar epithelial cells, in which myelin globules may be found inclosed. Occasionally eosinophiles are seen in large numbers, but this is less common than in asthma (which see).

The Urine.—The urine shows no changes which can be referred to the emphysema *per se*, but as cardiac and renal lesions develop in many cases in the later stages of the disease the urinary picture will at that time depend upon these complications. There is then usually more or less marked oliguria with high color, increased specific gravity, and urate sediments. There is frequently a small amount of albumin, associated with a few hyaline casts; occasionally a few red cells and a few leukocytes are seen.

ENDOCARDITIS (ULCERATIVE)

Essential Factors.—Secondary anemia; (in some of the cases) hyperleukocytosis with septic factor; presence of the corresponding bacteria in the blood.

The Blood.—*The Red Cells and Hemoglobin.*—In all cases of malignant endocarditis marked anemia develops sooner or later. Often it is very noticeable already quite early in the disease (after the second week), when counts of 3,000,000 to 3,500,000 are common. In cases of longer duration it develops more slowly. As the disease progresses it becomes more and more intense, and *sub finem vitæ* not more than 1,000,000 red cells may be counted. The loss of hemoglobin keeps step with the loss of the red cells and frequently exceeds it to a certain extent, so that a lowered color index develops.

In well-advanced cases the individual red cells are very pale, pessary forms being seen everywhere; typical blood shadows even may be encountered. Polychromatophilia is common. An increased vulnerability of the red cells to mechanical insults is very evident, as is shown by the occurrence of numerous corpuscles with creased, distorted contours, while poikilocytes proper are scanty. The size of the red cells is not increased; on the contrary, if any change in size be apparent it is rather in the direction of a decrease. Stiple cells do not belong to the blood picture of malignant endocarditis; an occasional one may be seen, but on the whole they are rare.

The Leukocytes.—While the leukocytes are unquestionably increased in many cases, others are met with in which the absolute counts show little or no deviation from the normal. A reason for this apparent incongruity is not always manifest. In some instances it may be due to an overwhelming intoxication, while in others this explanation can hardly hold good. Much, no doubt, depends upon the nature of the offending organism. In Cabot's series of 26 cases the initial count was 10,000 or more in 20, and higher than 15,000 in 15; the lowest was 3000 and the highest 34,000. Krebs noted an antemortem leukocytosis of 44,200, and Grawitz one of 168,000; in others a preagonal leukopenia develops. In some instances remarkable fluctuations are observed from day to day, normal values alternating with high ones. Systematic differential counts in large series are unfortunately not available. When a hyperleukocytosis exists this is apparently always referable to an increase of the neutrophiles, which is associated with a decrease or absence of the eosinophiles; in other words, we have the septic factor typically developed. Findings in those cases in which the leukocyte count remains normal are not available.

The Serum.—Hemoglobinemia is demonstrable in many cases.

Bacteriological Examination of the Blood.—This reveals the presence of the offending organism in a large percentage of cases. Lenhartz obtained positive results *intra vitam* in 16 cases out of 28, and Libman, whose experience in this direction is most extensive, states that in cases of ulcerative endocarditis he has always found organisms in the blood. The bacteria which have been encountered are the Staphylococcus aureus, streptococci, pneumococci, and the gonococcus. Of

these, the streptococcus cases are the most common; the staphylococcus comes next, while pneumococcus and gonococcus endocarditis are relatively uncommon. Libman remarks that there is often a marked disproportion between the number of bacteria in the blood and the extent of the lesion. There may be an almost countless number and only very small deposits on the valves, or there may be large vegetations with hardly any bacteria in the blood. As a rule, they are present in fair numbers, and can be demonstrated quite early in the disease. Positive findings may alternate with negative results, and repeated examinations may hence be necessary. Ewing writes that when malignant endocarditis follows the type of a pure septicemia with the cardiac symptoms in the background, bacteriological examination of the blood is usually positive, while this is negative when the cardiac symptoms are or have been prominent.

The Urine.—Examination of the urine shows no characteristic features; in a general way the findings resemble those seen in typhoid fever. The diazo reaction, however, is usually absent. The urea is greatly reduced in some cases, and especially toward the fatal end. Albumin is found in traces in about 42 per cent. of the cases, and may be abundant in about 14 per cent.; the amount increases as the disease progresses; coincidently there are hyaline and granular casts and an increased number of leukocytes.

ENTERITIS ACUTA

(Acute inflammatory diarrhea; enterocolitis; ileocolitis)

Essential Factors.—Secondary anemia with relative polycythemia; frequent hyperleukocytosis and lymphocytosis; presence of agglutinins in infections with the dysentery bacillus; vomiting; diarrhea; oliguria; irregular indicanuria and albuminuria.

The Blood.—In acute enteritis a certain degree of *secondary anemia* is almost invariable. Clinically this is usually very manifest, while the numerical findings, owing to a more or less extensive concentration of the blood, are apt to give an erroneous idea of its extent; not infrequently a polycythemia is thus observed instead of an oligocythemia. In a considerable number of cases the *leukocytes* are found increased, the extent depending upon the severity of the intoxication and the degree of the blood concentration; 10,000 to 15,000 are common values; less frequently still higher figures—up to 30,000—are encountered. The differential count gives variable results. When hyperleukocytosis exists, referable to blood concentration merely, the percentage values of the different forms may remain undisturbed. In many cases, however, a well-marked lymphocytosis either relative or absolute is observed. In children this is particularly marked. In the *summer diarrhea* of infants the results are variable and require

renewed investigations, more particularly in reference to the type of infection, the existence of complicating conditions, etc. A neutrophilic hyperleukocytosis may be expected when the enteritis is a complicating factor of an infection which in itself calls forth a neutrophilic increase. Wochnert cites a case of subacute colitis following an attack of influenza pneumonia with a total count of 14,000 and 97 per cent. of lymphocytes.

Serum Diagnosis.—The serum diagnosis of infections with the dysentery bacillus has been attempted by several investigators. Shiga claimed originally that his bacillus was clumped only by the serum of patients with the corresponding infection. This is not strictly true, since the reaction has at times been obtained with non-dysenteric patients. The test has a certain value, nevertheless, and may be used in the selection of those cases of summer diarrhea of infants especially in which treatment with antidysenteric serum is to be instituted. Pilsbury states that he has not met with false positive reactions in the blood of non-dysenteric infants under one year of age.

The bacteriological diagnosis of the various types of enteritis from an examination of the blood is not practical, even if at times it may be possible.

The Stomach Contents.—Vomiting is a common initial symptom in all the different types of enteritis, and may continue throughout the course of the disease. In other cases it ceases when the intestinal symptoms have entered prominently into the foregound. The chemical findings are essentially those of an acute gastritis (which see).

The Feces.—Diarrhea is a prominent symptom in practically all cases. The number of the stools is in a general way an index of the intensity of the morbid process, and may vary from five to six to twenty or even thirty or more in the twenty-four hours. The material at first is pultaceous and represents the ordinary contents of the lower bowel. Very soon, however, the movements become watery, more frequent, and smaller in amount, excepting in particularly severe infections where the discharges are copious throughout. The color is usually a light or dark brown. In infections with the bacillus of Le Sage it may be grass-green. Mucus is present in variable amount; when the colon is involved it is usually abundant and may be seen in the form of tapioca-like particles. In severe cases there is more or less blood and pus. The odor is usually not very offensive, but may become very bad when gangrenous processes supervene. Microscopic examination reveals the presence of enormous numbers of bacteria, leukocytes, and epithelial cells in various stages of degeneration, blood corpuscles, debris, not infrequently triple phosphate crystals, and in severe ulcerative cases smaller or larger shreds of necrotic material.

On bacteriological examination the offending organisms may be demonstrated, but for purposes of diagnosis these methods are rarely applicable. The list includes the colon bacillus, the Bacillus lactis

aërogenes, the dysentery bacillus, the bacillus of Finkler and Prior, the green bacillus of Le Sage, the Bacillus pyocyaneus, etc.

The Urine.—Owing to the losses of water through the bowels, and deficient ingestion, there is of necessity a marked oliguria in all cases of acute enteritis. The specific gravity is more or less increased, and on standing deposits of phosphates or urates readily form. In some instances there is increased indicanuria. The sum of the conjugate and mineral sulphates, as well as the latter, is diminished while the ratio of A to B is increased. Acetone and diacetic acid have been repeatedly found. Albuminuria and cylindruria may be met with in severe cases, and exceptionally actual nephritis may develop.

ENTERITIS CHRONICA

Essential Factors.—Irregular secondary anemia, at times with relative polycythemia; lymphocytosis; diarrhea, alternating in some cases with constipation; mucorrhea; presence of animal parasites in certain cases.

The Blood.—The blood picture in chronic enteritis is quite variable. In many cases there is no material deviation from the normal, while in others a marked *secondary anemia* develops which is clinically quite manifest, whereas, the numerical findings owing to concentration of the blood often give an erroneous impression of the existence of normal conditions. The greater the tendency to diarrhea, *cæteris paribus*, the greater is the tendency to anemia.

Leukocytosis is variable. In most cases normal absolute values are met with, while lymphocytosis either relative ar absolute is common.

The Feces.—In all cases there is diarrhea, which may persist as such or alternate with attacks of constipation, or periods of fairly normal movements. When diarrhea exists the appearance of the stools both macroscopically and microscopically does not differ materially from what is seen in acute enteritis. On the whole, however, watery evacuations are less common; in many cases the movements are a thin mush. The amount of mucus varies, but is usually in proportion to the extent to which the colon is involved. During periods of constipation scybalous masses may be passed which are densely coated with mucus, and in a certain class of cases large amounts of the material may be passed as such—*enteritis membranacea* (*colica mucosa*). In some instances the mucus appears in the form of ribbons or casts of the colon, which may be a foot or more long—the so-called *mucus cylinders*, while at other times it forms a jelly-like mass, which may be sufficiently abundant to fill a tumbler.

Bacteriological Examination.—The bacteriological examination in chronic enteritis rarely furnishes information of value. Of great interest, on the other hand, are those cases in which infusoria can be demonstrated. The organisms in question are the Balantidium coli,

the Cercomonas intestinalis, and the Trichomonas intestinalis. All of these unquestionably can keep up a diarrhea, even if they have not been the original causative agent. Of the larger parasites, the hookworm at times sets up a chronic enteritis, while in certain countries the Strongyloides intestinalis is a notorious agent in this respect; the presence of a fair amount of blood is a common symptom in infections of the latter kind. In the hookworm cases the corresponding eggs will be found, while in the strongyloides infections the embryonic worms are encountered.

The Urine.—The urine shows no material deviation from the normal; when there is much diarrhea there is corresponding oliguria. In some instances indicanuria is observed, but this is inconstant. Albuminuria and cylindruria do not belong to the picture, unless the patient's health has been very seriously undermined.

EPILEPSY

Essential Factors.—Chlorotic anemia; irregular hyperleukocytosis with relative lymphocytosis; irregular postepileptic albuminuria; normal cytological formula of the meningeal fluid.

The Blood.—According to Boston there is in idiopathic epilepsy a mild degree of *chlorotic anemia.* His red counts in seven specially selected cases ranged between 4,610,000 and 5,620,000 and the hemoglobin values from 63 to 86 per cent. Similar results have been recorded by Smyth.

The Leukocytes.—The leukocytes were increased in all but one, in which the count was 7400. The highest value was 18,000. In three cases the differential count showed a marked diminution of the polynuclears, viz., to 29, 42, and 43 per cent. Boston remarks that this reduction was not associated with an absolute increase of the lymphocytes. Myelocytes were found in three instances, with a maximum of 3.5 per cent. Kuhlmann, in contradistinction to Boston, found a leukocytic increase in only 1 case out of 16. Itten reports the leukocyte count low in the interval and a tendency to increase shortly before and after the attack. Before and during the attack he found a lymphocytosis, followed by a neutrophilic hyperleukocytosis. These findings he regards as of considerable diagnostic value, since hysterical and paralytic seizures are not associated with a primary lymphocytosis. Following the injection of crotalin which has been advocated for the treatment of epilepsy there is a notable increase of the eosinophiles (35 per cent.).

Serology.—Using brain tissue as antigen, Wegener obtained a positive Abderhalden reaction only in cases in which dementia existed, while Binswanger concludes that a positive reaction in the interval justifies the assumption that progressive changes are taking place in the cortex. Leri and Vurpas find that the reaction is apparently

not influenced by the number or severity of the attacks. Most of the positive reactions were obtained in patients presenting outspoken mental deterioration.

The Urine.—The urine shows no special abnormality in the interval, while immediately after an attack there may be slight albuminuria, which in some cases at least is due to loss of seminal fluid during the convulsive seizure. Sigmund noted a transitory glucosuria in 7.4 per cent. of his epileptic cases, and various older writers speak of the occurrence of glucosuria after attacks. An analysis of the data of these latter has led me to the conclusion that their inferences are scarcely justifiable, as satisfactory proof of the presence of sugar has not been furnished. During epileptic seizures, in contradistinction to attacks of major hysteria, there is said to be an increased elimination of phosphates.

The Cerebrospinal Fluid.—The amount of fluid which may be obtained from epileptics is, according to Pellagrini, quite small, viz., 10 to 15 c.c., while Donath gives much higher figures—up to 60 c.c. The latter claims to have isolated cholin from the fluid in 15 cases out of 18. Cytological examination does not reveal any deviation from normal conditions. Pellegrini believes to have demonstrated that the meningeal fluid of epileptics is markedly toxic and that the material obtained directly after a convulsion has a toxic and convulsive power which is much greater than that obtained at intervals far removed from paroxysms. Similar results are reported by Dide and Laquepée.

ERYSIPELAS

Essential Factors.—Hyperleukocytosis with septic factor; irregular presence of streptococci in the blood; irregular bacteriuria.

The Blood.—*The Red Cells and Hemoglobin.*—A certain degree of anemia develops in all cases of erysipelas, and is, generally speaking, proportionate to the intensity of the infection and the duration of the disease. It is of the chlorotic type and usually does not exceed a loss of 30 per cent. of the hemoglobin and of 10 to 20 per cent. of the corpuscles. In mild cases it is insignificant.

The Leukocytes.—The leukocytes are increased in nearly all cases, the degree of hyperleukocytosis being proportionate to the intensity of the morbid process. In some cases the number scarcely exceeds the upper limit of the normal, while in the severer types it may reach 20,000 to 30,000 or more. Normal values may be met with in isolated cases, but constitute the exception. In cases which terminate by crisis the hyperleukocytosis may also disappear abruptly, the drop sometimes occurring several hours before the fall in temperature. In lytic cases the return to the normal is more gradual. The differential count reveals the septic factor, viz., an increase of the neutrophiles associated with a decrease or absence of eosinophiles;

this disappears with the decline of the temperature, and during convalescence an epicritic eosinophilia is common, which may reach 10 per cent. At the height of the disease the neutrophiles are frequently between 80 and 90 per cent., and in very severe cases the number may be still higher. A few myelocytes are commonly seen, and during convalescence they may be temporarily quite numerous, reaching 6 to 8 per cent.

Some writers remark that in children the lymphocytes are increased at the height of the process, but of this I have no personal knowledge.

During the active stage of the disease a few phlogocytes may be met with.

The Plaques.—The plaques are increased in severe cases. The same holds good for the quantity of *fibrin.* The alkalinity is said to be diminished, but I do not think that the available data on this point are reliable.

Bacteriological Examination.—Bacteriological examination of the blood may or may not reveal the presence of the offending microorganism. In most cases the culture is negative.

Generally speaking, death is most apt to occur in those in which a bacteriemia has developed.

The Urine.—The urine shows the usual features of an acute febrile process. In cases of ordinary severity no material abnormalities occur, but in severe cases albuminuria and cylindruria are common. Sugar is absent. When nephritis develops, bacteriuria probably always accompanies the condition, and with the cessation of the disease both disappear together.

ERYTHROMELALGIA

In several cases of this disease the blood findings have been essentially the same as those described under the heading of enterogenous cyanosis.

FILARIASIS

Essential Factors.—Hyperleukocytosis and eosinophilia early in the disease; subsequently normal values; general tendency to lymphocytosis; presence of filaria embryos in the circulation; chyluria; chylous exudates; chylous diarrhea.

The Blood.—*The Red Cells and Hemoglobin.*—In cases where the patient's general condition suffers as a result of chyluria, ascites, diarrhea, and similar complications, anemia of greater or less degree is a common symptom. Where this does not occur the red count and hemoglobin values will be found practically normal.

The Leukocytes.—Various writers report that early in the disease there is hyperleukocytosis, but subsequently normal numbers are the rule, unless inflammatory complications supervene. Calvert states that coincidently with the primary increase of the total number

an increase of the eosinophiles also may be looked for, but that these return to normal as the disease progresses. In one of his cases the percentage rose to 22, but varied within twenty-four hours between this point and 8. In one case of long standing with but few parasites I found 2 per cent. of eosinophiles with 36 per cent. of lymphocytes. Calvert likewise noted a lymphocytosis. A relation between the numbers of embryos and the percentage of the different leukocytes does not exist.

Presence of Filaria Embryos.—The diagnosis of filariasis depends, of course, upon the demonstration of the corresponding parasites. Only the embryonic worms find their way into the peripheral circulation. According to Manson at least four, and possibly five, or even more distinct species enter into consideration, viz., the Filaria nocturna, F. diurna, F. perstans, F. demarquaii, F. ozzardi (a doubtful species), and a sixth which may or may not be connected with one of the two last, the F. magelhæsi. Two of these at least are of pathological import, viz., the F. nocturna and the F. perstans. F. nocturna is the embryonic form of F. bancrofti which inhabits the lymphatics and is unquestionably the cause of endemic chyluria, of various forms of lymph varix, of tropical elephantiasis arabum and possibly also of other obscure tropical diseases.

The number of worms which may be found in a specimen is variable. During the daytime they are rarely seen, and, if at all, only one or two specimens at most are found. As evening approaches, however, commencing about five or six o'clock, the filarias enter the peripheral circulation in increasing numbers. At midnight the maximum is about reached, with from 300 to 600 to the drop of blood. Later they gradually decrease, and by 8 or 9 A.M., they have again disappeared. This periodicity may be reversed if the patient is made to sleep during the daytime and remains awake at nights. During their absence from the peripheral circulation they may be found in the larger arteries and in the lungs.

In non-active cases the number of filarias even at night is quite small. In one instance of this kind I found only the sheath of a single worm while examining perhaps fifty specimens.

According to Manson the number of filarias is smaller in the elephantiasis cases than in those which are not affected in this manner. This he explains on the basis that in the former a larger area of the lymphatic system is blocked than in the latter, and coincidently a lesser likelihood of an unobstructed passage to the blood.

The Filaria perstans, unlike the F. nocturna, observes no periodicity, but is present in the blood both during the daytime and at nights.

The Urine.—This shows no special abnormalities unless a lymph varix in the walls of the bladder, the consequence of filarial obstruction in the thoracic duct, ruptures with consequent occurrence of

chyluria. Sometimes this is preceded by retention. The following description of the condition is taken from Manson: If chylous urine be passed into a urine glass and allowed to stand, within a very short time, as a rule, the whole of the urine becomes coagulated. Gradually the coagulum contracts until, at the end of some hours, a small more or less globular clot, usually bright red or pinkish in color, is floating about in a milky fluid, Later the milky fluid separates into three layers. On the top there is formed a cream-like pellicle; at the bottom a scanty reddish sediment, sometimes including minute red clots; in the centre the mass of the urine forms a thick intermediate stratum, milky white or reddish white in color, in which floats the contracted coagulum. If a little of the sediment be taken up with a pipette and examined with the microscope, it is found to contain red blood cells, lymphocytes, granular fatty matter, epithelium and urinary salts, and, mixed with these in a large proportion of cases, though not in all, filaria embryos. The middle layer contains much granular fatty matter; while the upper cream-like layer consists of the same fatty material in greater abundance, the granules tending to aggregate into larger oil globules. If a small portion of the coagulum be teased out, pressed between two slides, and examined with the microscope, filariæ, more or less active, may be found in the meshes of the fibrin. If ether be shaken up with the milky urine, the fat particles are dissolved out and the urine becomes clear; the fat may be recovered by decanting and evaporating the ether which floats on the urine. Boiling the urine throws down a considerable precipitate of albumin.

Chyluria comes and goes in a very capricious manner. Sometimes the urine remains steadily chylous for weeks and months, and then suddenly, without obvious cause, becomes limpid and natural looking and free from fat or albumin. Later a relapse will occur, to disappear again after an uncertain time; and so on during a long course of years.

Retention of Urine.—Retention of urine is not an unusual occurrence; it is produced by the formation of a coagulum in the bladder. The retention usually gives way after a few hours of distress, worm-like clots being passed.

Exudates.—In cases of chylous dropsy of the tunica vaginalis (chylocele), enormous numbers of embryos may be found in the chylous fluid. In rare instances there is chylous ascites and chylous diarrhea. (For a description of the parasites in question see Filaria in the first section of the book.)

FOOT-AND-MOUTH DISEASE

In one instance of this disease occurring in the human being reported by Clough, a total leukocyte count of 10,000 was noted on

the sixth day of the disease, with the following differential values: small monos 12, large 12, polynuclear neutrophiles 73, eosinophiles 1, and basophiles 1.

FRACTURES

The Blood.—From a study of 38 cases, Cabot, Hubbard, and Blake conclude that the blood shows no abnormalities. Of these, 23 were simple fractures and 15 complicated cases. In the former the count exceeded 10,500 in 10 and 12,000 in 6; the highest count was 15,400 in a fracture of the pelvis. In the complicated cases a definite hyperleukocytosis was noted in only 2, viz., 15,600 in a fracture of the tibia and fibula with symptoms pointing to fat embolism, and 14,900 in a case of fractured ribs with injury to the lung. Differential counts are unfortunately not reported.

In one case of compound fracture of the ankle with consequent cellulitis I found a blood picture which was typical of myelocytic leukemia, with large numbers of neutrophilic myelocytes (15 per cent.), some eosinophilic myelocytes, and about 5 to 6 per cent. of mast cells; coincidently a moderate number of normoblasts was found. After a few days there was a return to the normal. On inquiry, Hastings wrote me that he had found myelocytes to the extent of 3 to 7 per cent. in cases of fracture.

In fractures of the long bones with injury to the fatty marrow lipemia has been observed.

The Urine.—The urine shows no special abnormalities in uncomplicated cases.

FRAMBESIA

According to Glogner, frambesia is associated with a more or less marked lymphocytosis.

Examination of the serum is said to show complement fixation with the usual antigens employed in the Wassermann test.

In the corresponding lesions the offending trepanoma (Spirochete pertenuis, Castellani) may be demonstrated. Their length varies between 7 and 20μ. They resemble the T. pallida, but their ends are sometimes a little rounded off.

GANGRENE (OF THE LUNG)

Essential Factors.—Secondary anemia; hyperleukocytosis of the neutrophilic type; putrid odor and trisedimentation of the sputum; presence of elastic tissue and fatty acid needles, and occasionally of cholesterin, leucin, and tyrosin.

The Blood.—*The Red Cells and Hemoglobin.*—In those cases in which gangrene develops secondarily to other pathological conditions

(fetid bronchitis, croupous pneumonia, catarrhal pneumonia, bronchiectasis, tuberculosis, carcinoma, etc.), the red count and hemoglobin will depend upon the underlying disease. In the primary cases, on the other hand, where gangrene follows the aspiration of foreign bodies, in otherwise healthy individuals there need be no deviation from the normal at the start. Sooner or later, however, anemia develops in all cases and may be quite extensive.

The Leukocytes.—In all active cases hyperleukocytosis must of necessity occur, while normal counts may be met with during periods of improvement. In the majority of cases no doubt there is a polynucleosis.

The Sputum.—The sputum shows the same general features which are seen in bronchiectasis. The most striking factor is the nauseating stench which pervades the whole atmosphere of the patient. This is observed in most cases where the disease is well developed, but at times there is scarcely any odor at all. According to Emerson this absence of fetor is seen particularly in diabetes, in the insane, and in gangrene from embolism. The quantity is usually considerable; not infrequently it amounts to from 200 to 500 c.c. Trisedimentation is generally perfect. The color of the material as a whole is a dirty greenish gray, when no blood is present; otherwise, and this is very frequently the case, it is additionally tinged in various shades of red and dirty brown. The diagnosis of the condition, so far as the sputum goes, rests upon the demonstration of constituents of lung parenchyma. Such material may vary in size from that of a pinhead to ragged pieces measuring several centimeters in length. According to Osler elastic tissue can be demonstrated in every case. Dittrich's plugs are occasionally also seen. Microscopically, one finds enormous numbers of bacteria, some pathogenic, others purely saprophytic; among the latter long threads of the Leptothrix pulmonalis. A number of writers have described certain acid-fast bacteria, which, however, are decolorized by acid alcohol. Sahli mentions a case in which sarcinæ were numerous, and several writers have described the occurrence of trichomonads and cercomonads, occurring both in the sputum as such, as also in the plugs of Dittrich. Well-preserved cellular elements are scanty. Fat globules and fatty acid needles are usually abundant, and occasionally one meets with cholesterin, leucin, and tyrosin. Any blood that may be present is commonly decomposed, or the hemoglobin changed to methemoglobin. Chemical examination shows the presence of tyrosin, leucin, ammonia, hydrogen sulphide, butyric acid. valerianic acid, caproic acid, etc. The reaction of the fresh material is usually alkaline, but on standing it usually becomes acid.

The Feces.—Considering the character of the expectoration, it is not surprising that many of the cases develop diarrhea, which no doubt is caused by the swallowing of some of the fetid material.

The Urine.—The urinary findings are essentially the same as in bronchiectasis, and depend to a great extent upon the nature of the underlying malady and its duration.

GASTRITIS ACUTA

Essential Factors.—Occasional neutrophilic hyperleukocytosis (?); motor insufficiency; absence or diminution in the amount of hydrochloric acid; presence of organic acids; oliguria and irregular indicanuria.

The Blood.—In acute gastritis there are no blood changes of moment, barring a variable degree of concentration of the blood, the result of vomiting, with a coincident *relative polycythemia* and occasional *hyperleukocytosis*. This latter may be of the neutrophilic type; satisfactory data on this point are, however, very meager, and it should not be forgotten that many cases of so-called acute gastritis are in reality something else, and the gastric symptoms reflex or secondary. Many cases of mild and moderately severe appendicitis are diagnosticated as gastritis, and I fear that some of the cases of "gastritis" in which hyperleukocytosis of the neutrophilic type has been observed may have been cases of appendicitis. *The diagnosis of acute gastritis is frequently a very difficult problem.*

The Stomach Contents.—Vomiting is one of the most constant symptoms. This may occur very soon after the causative factor in question has become operative, or it may be delayed for a number of hours. Frequently food remnants from a previous meal are brought up. The amount is abundant, the food components insufficiently digested or scarcely affected at all and mixed with a large amount of mucus. The material shows an acid reaction which is largely referable to the presence of organic acids (lactic, butyric, and acetic acid), while free hydrochloric acid is usually absent and the total acidity diminished. As a result of the violent retching small amounts of bile are frequently present.

Examination after the administration of the test breakfast and previous vomiting or lavage shows very feeble digestion of the bread and usually absence of free hydrochloric acid with low total acidity values.

The Urine.—The urine during the attack and shortly thereafter is much diminished in amount and is apt to deposit a sediment of urates. At times there is increased indicanuria and in severe cases a trace of albumin may appear with a few hyaline casts. With convalescence the amount increases, the color becomes lighter, and the tendency to form deposits of urates disappears.

GASTRITIS CHRONICA (NON-MALIGNANT)

Essential Factors.—General tendency to moderate chlorotic anemia with relative polycythemia; normal leukocytosis or leukopenia; hyperchlorhydria or anachlorhydria; variable motor power.

The Blood.—*The Red Cells and Hemoglobin.*—The blood shows no material changes in chronic gastritis, unless the disease has existed for a long time and has affected the patient's general nutrition. Even then, probably owing to a concentration of the blood, the numerical findings are often practically normal. The hemoglobin estimation is more apt to give an idea of the extent of the anemia than the red count. In Lichty's series of fourteen cases of chronic gastritis the average red count was 5,498,000 and the average hemoglobin value 91 per cent.

The Leukocytes.—The leukocytes are not increased, and may, indeed, be diminished. The differential count may show a moderate lymphocytosis. Occasionally there is no digestive leukocytosis.

The Stomach Contents.—Vomiting is the exception. When it occurs, the food material which has been brought up shows evidence of imperfect digestion. Often there is a considerable admixture of mucus. In alcoholic cases especially morning vomiting of neutral or feebly acid mucus with small amounts of bile is a common event (vomitus matutinus).

Following the administration of a test meal the contents of the stomach show little or no digestion at a time when under normal conditions digestion should already be well advanced. There is an intimate admixture of mucus, which frequently renders the removal of the stomach contents and their filtration a difficult matter. Generally speaking, the amount of mucus is inversely proportionate to the amount of hydrochloric acid. The largest amount is found when this is absent. In the majority of cases of chronic gastritis the secretion of hydrochloric acid is more or less impaired, the deficiency being ascertained by titration with decinormal hydrochloric acid (which see). In extreme cases the secretion of gastric juice ceases entirely (achylia gastrica, which see). Rarely one observes an increased production of hydrochloric acid in association with an increased production of mucus—*gastritis acida.* The loss of hydrochloric acid is accompanied by a loss in pepsin and in chymosin, which, however, is usually less extensive. Boas has emphasized that the estimation of the chymosinogen is of special importance in gauging the extent of the damage and in determining the prognosis. The nearer the amount of zymogen approaches the normal the greater will be the probability of ultimate recovery under suitable treatment. When it is greatly diminished or absent altogether the condition is usually incurable.

In some cases lavage reveals the presence of small bits of mucosa, which may be used for a histological examination.

The Motor Power of the Stomach.—This may be quite variable. Frequently it is normal or even increased. In other cases, owing to muscular atony or benign obstruction at the pylorus the propulsion of the food is delayed. In such cases fermentation and gas production may be observed.

The Feces.—Usually there is constipation, occasionally constipation alternating with diarrhea, more rarely diarrhea alone.

The Urine.—In cases associated with marked motor insufficiency there is usually oliguria of greater or less extent with high specific gravity and a tendency to the deposition of phosphates. Indicanuria is usually marked. Otherwise no special abnormalities are noted.

GASTROSUCCORRHŒA ACIDA

(Chronic hypersecretion; hypersecretio acida et continua)

Essential Factors.—Relative polycythemia in cases of long standing, associated with actual anemia; continuous secretion of acid gastric juice irrespective of the introduction of food; hyperchlorhydria; motor insufficiency (pyloric spasm) with frequent evidence of dilatation; vomiting of large quantities of acid gastric juice at nights; oliguria; decrease in the acidity of the urine and the chloride content.

The Blood.—Early cases of chronic hypersecretion show no abnormality of the blood picture. After the disease has persisted for a long time there may be marked anemia, so far as the appearance of the patient goes, which is largely obscured, however, numerically through a concentration of the blood. As in other conditions of a similar nature, the hemoglobin estimation gives a better, though also an imperfect idea of the degree of anemia than the red count. The leukocytes are either normal or moderately increased from the same cause.

The Stomach Contents.—The diagnosis of the condition, aside from the clinical history, depends upon the demonstration of notable gastric secretion by the fasting organ. In suspected cases it is necessary to wash the stomach clean the evening before, to allow neither food nor drink thereafter, and to siphon off the contents the next morning. This is necessary in order to eliminate confusing factors which would enter into consideration, when, as frequently occurs, the succorrhea is associated with marked motor insufficiency and dilatation. In cases of succorrhea the fasting organ will be found to contain from 100 to 500 c.c. of fluid, in which notable amounts of hydrochloric acid can be demonstrated, while organic acids are absent. Occasionally the amount is still larger, viz., 1000 c.c. or more. After the administration of a test meal large amounts of material will similarly be obtained, and when vomiting occurs one is struck with the excessive quantities brought up, as compared with the amount corresponding to the meal ingested. Going hand in hand with the hypersecretion there is usually also a corresponding

hyperchlorhydria with total acidity values of 50 to 80 or more. Pepsin is always present and proteolysis accordingly very active. Amylolytic digestion, on the other hand, is seriously interfered with and there is accordingly a marked tendency to carbohydrate fermentation and gas formation referable to the action of yeast cells, which can usually be demonstrated in large numbers on microscopic examination. This is, of course, the more marked the more extensive the motor insufficiency. Sarcinæ likewise are common.

Vomiting.—Vomiting is a common symptom in chronic hypersecretion and especially characteristic when it takes place at hours of the night when the stomach should be empty. It is surprising to see what large amounts of fluid may thus be brought up; albuminous food remnants are usually lacking, while starchy food can frequently be demonstrated either macroscopically or on microscopic examination. On standing marked trisedimentation occurs, viz., an upper frothy layer, a middle turbid layer representing the main bulk of the material, and a lower layer, which is largely composed of undigested starchy food. At times there is a slight admixture of bile and not infrequently of blood. The occurrence of actual hemorrhage indicates the development of an ulcer—a not infrequent complication.

The Urine.—Corresponding to the frequently very considerable loss of fluid through vomiting, there is a diminution in the amount of urine, which may amount to one-half of the normal amount. The specific gravity is increased, and on standing a deposit of phosphates is apt to separate out. As one would expect, the acidity as well as the chlorides are markedly reduced. Other abnormalities have not been observed.

GAUCHER'S DISEASE

The Blood.—The blood picture depends upon the stage of the disease. Generally there is anemia of the secondary type of variable extent. The leukocytes are usually reduced in number and may fall as low as 500 per c.mm. Normal values may be found, but an analysis of the reported cases shows a distinct tendency to leukopenia. The differential count is usually normal.

GERMAN MEASLES

(Rötheln; rubella)

The Blood.—The blood findings in German measles are essentially the same as those in true measles. There is no hyperleukocytosis. In a few cases which I had occasion to examine there was a lymphocytosis of moderate grade and normal eosinophilia. The red count and the blood platelets remain unaffected.

The Urine.—The urine shows nothing characteristic.

GLANDERS

Essential Factors.—Presence of the bacillus mallei in the discharge from the nose, and in the contents of pustules and abscesses.

The Blood.—Nothing is known regarding the red count and hemoglobin values, while several observers report that the leukocytes are increased.

Duval and v. Jaksch claim to have isolated the corresponding organism (B. mallei) from the blood during life, while other observers have not been successful in this respect.

Heanly states that the patient's serum will agglutinate the organism in a dilution of 1 to 2500 in twelve hours, while with lower dilutions a positive result may also be obtained with the serum both of scarlet fever and smallpox patients.

The Pus.—In the pus of glanders abscesses and in the contents of corresponding *pustules* the characteristic bacillus may be demonstrated. It may also be obtained from the purulent discharge of the nose which occurs in nearly all cases.

The Urine.—The urine at times contains a small amount of albumin and in rare cases there is icterus with choluria.

GONOCOCCUS INFECTIONS

Essential Factors.—Neutrophilic hyperleukocytosis with normal or increased eosinophile values; gonococcemia; complement fixation; presence of the gonococcus in the urethral discharge; pyuria; presence of gonorrheal threads.

The Blood.—*The Red Cells and Hemoglobin.*—In ordinary cases of gonorrhea no anemia of note develops; if complications supervene, however, and especially if the infection becomes generalized, extensive destruction of the red cells may take place. In gonorrheal endocarditis anemia is accordingly always a prominent symptom. Usually the red count is below 2,000,000 and at times the oligocythemia may be so extensive as to suggest pernicious anemia; Osler has reported two instances of this kind. The loss of hemoglobin is apt to exceed the loss in corpuscles, so that a lowered color index results. The morphological changes are then identical with those seen in ordinary cases of septicemia.

The Leukocytes.—Mild cases of gonorrheal urethritis show no material increase in the number of the leukocytes; the severer forms, however, and those complicated with epididymitis, orchitis, cystitis, etc., are associated with hyperleukocytosis. This may or may not be extensive. The endocarditis cases show values which usually range between 8500 and 18,000. In the pure gonococcus infections showing an increase of the leukocytes this is referable to the polynuclear

neutrophiles, but contrary to what we see in infections with the common pus organisms the neutrophilic increase is rarely associated with a decrease of the eosinophiles. Sometimes these are actually increased, and if not, their number is at least normal. In Bettmann's series the values ranged between 0.5 and 11.5 per cent.; the highest figure (25 per cent.) was obtained in a case of gonorrheal epididymitis. From an analysis of 45 cases which Owings studied in my laboratory, it appears that, with an extension of the inflammatory process to the posterior urethra, the number of cases increases in which an increased percentage of eosinophiles is found in the blood, and that in cases of prostatitis this is the rule. Owings' results are, in this respect, identical with those reached by Bettmann. Regarding the time at which the eosinophilia appears Owings came to the conclusion that during the first week no deviations from the normal occur. During the second and third week a normal count was obtained in only one-third of the cases, while after two months' duration an increased number of eosinophiles was still noticeable in 40 per cent. of the cases.

Several observers have reported a marked iodophilia in gonococcus infections, which may aid in the diagnosis of the arthritis cases. In the latter the eosinophiles are also said to be increased.

In those cases in which no hyperleukocytosis occurs the neutrophiles are frequently diminished, while the lymphocytes and often also the large mononuclear leukocytes are increased.

Differential counts in gonococcus endocarditis are available only in a very small number of cases; apparently no deviation from the normal need occur. Jacob cites an instance with 34.5 per cent. of mononuclear elements, 62 per cent. of neutrophiles, 1 per cent. of eosinophiles, and 2.5 per cent. of mast cells.

Bacteriology.—In systemic infections the gonococcus may be cultivated from the blood in a large number of cases. Thayer-Blumer, Thayer-Lazear, Byelogoway, Wilson, Harris-Johnston, and many others have isolated the organism in cases of gonorrheal endocarditis. Ahmann, Colombini, Panichi, and Unger obtained positive results in cases of gonorrheal arthritis, epididymitis, myositis, tendovaginitis, inguinal bubo, and parotitis. In the endocarditis cases cultures were obtained after an illness lasting for from five to seven weeks to seven months, at times as early as the ninth to the eleventh day preceding death, and on an average five days before death. Moore, of the U. S. Marine Hospital Service, could demonstrate the organism directly in a blood smear, in a fatal generalized infection.

Serology.—The gonococcus complement-fixation test furnishes a positive result in a large per cent. of the chronic cases, while it is commonly negative during the acute stage of the disease. It unquestionably is the most important method of determining whether the disease has been eradicated, or whether a residual infection still exists in the body. Of O'Neill's series of 119 urethral cases

which were examined with the complement-fixation test but 109 were clinically uncured; of these, 95 were positive and 14 negative. Of 27 cases with a doubtful cure 9 were positive and 18 negative. Schwarz and McNeil, as the result of their study of a large number of cases of acute and chronic gonococcus infection of the most divers types, conclude that the method will have a definite place in the field of clinical pathology as an aid in the differential diagnosis between the various chronic conditions arising from gonorrheal infection and similar conditions arising from other causes. Of their 13 cases of gonorrheal arthritis 1 reacted in a positive manner; of 7 cases of doubtful origin 4 were positive and 3 negative; of 36 cases of chronic urethritis in which the gonococcus could no longer be demonstrated 27 were positive. Of 25 cases of chronic prostatitis with a gonorrheal history but without organisms in the discharge, 17 were positive. Very interesting, also, are the findings in clinically cured cases of gonorrhea. Of 52 cases of this order 22 still gave a positive reaction, which naturally raises the question whether these cases can be regarded as actually cured, or whether foci of living gonococci were still present somewhere in the body. Similar results have been obtained by other observers. Thomas and Ivy obtained positive reactions in 9.9 per cent. of anterior and posterior urethritis; in 21.5 per cent. of clinically cured cases; in 66.26 per cent. of stricture cases; in 87.5 per cent. of epididymitis, and in 100 per cent. of gonorrheal arthritis. (The report is based upon a study of 240 cases of gonorrheal disease.) To judge from what we know regarding the persistence of antibodies in the blood following immunization experiments in animals the inference would seem justifiable that a persisting complement fixation would indicate a persisting infection.

Exudates.—In a *pleuritic effusion* occurring in a case of gonorrheal septicemia with endocarditis Jacob has reported the following cytological formula: Lymphocytes, 22.4; endothelial cells, 22.4; neutrophiles, 5; eosinophiles, 29.4; and mast cells, 24.6 per cent.

Gonorrheal Pus.—Early in the disease, in acute cases, the morphological elements of the urethral discharge are principally polynuclear neutrophilic leukocytes; in addition there are small numbers of lymphocytes and large mononuclear leukocytes, a few eosinophiles and pavement epithelial cells; the iodophilia of the neutrophiles is then slight. The number of gonococci at this time varies with the intensity of the malady; the majority are found inclosed in the neutrophilic elements. From the sixth to the tenth day the iodine reaction becomes more marked, while the neutrophiles control the morphological picture almost entirely. From the second to the fourth week, as the discharge lessens, the number of both neutrophiles and gonococci diminishes; the lymphocytes and eosinophiles, on the other hand, increase, and with these the epithelial cells; the iodin reaction at this time is well marked. In subacute conditions the cytological

picture is essentially the same. In the chronic cases (secondary infection) the discharge, which was at first pure creamy pus, becomes mucoid and often appears as a "morning drop." The gonococci are then usually very scanty, and may, indeed, be absent; the polynuclear neutrophilic elements still occupy the foreground, but lymphocytes, large mononuclear leukocytes, and epithelial cells are likewise present in large numbers, while eosinophiles are only occasionally seen. Mast cells may be present, but in many cases they are absent. Neisser has reported a case in which the pus consisted almost exclusively of these elements.

The neutrophiles in gonorrheal pus commonly present evidence of degeneration. In some a loss of granular material has manifestly taken place, and it can be demonstrated that in most of the cells the granules are no longer absolutely neutrophilic, but have become amphophilic—that is, from a neutral mixture they take up the neutral dye, but they can also be stained with acid dyes.

As regards the distribution of the gonococci, these are found almost exclusively in the polynuclear neutrophiles; but occasionally they may be seen in some of the large mononuclear leukocytes; in the small mononuclear leukocytes and eosinophiles they are not found. Regarding the number of the latter, it sometimes appears that they are particularly numerous in cases where the gonococci are scanty and *vice versa*, from which fact some observers have drawn the inference that their presence is the expression of a successful defensive reaction and hence of prognostic significance. Other writers, however, report having found large numbers of eosinophiles associated with large numbers of cocci. Some of the eosinophiles which are found in gonorrheal pus are polynuclear and conform in their general appearance to the eosinophiles of the blood; others are mononuclear, and probably histogenic in origin.

The Urine.—In uncomplicated cases of anterior gonorrheal urethritis the urine shows no material deviation from the normal, beyond the presence of a variable amount of *pus* which appears in the first portion that is voided, while the second is clear. The first portion may accordingly also contain a trace of albumin which is referable to disintegrated leukocytes and exuded serum. If the urethritis extends to the neck of the bladder the first portion is, of course, also turbid, while the second may present a variable appearance, being clear at times and cloudy at others, when pus has found its way into the bladder. When cystitis complicates the urethritis, the second portion contains at least as much pus as the first and usually more, owing to the fact that the pus settles to the bottom and is voided at the end of micturition; the last drops in such cases may consist of pure pus.

In the later stages of acute gonorrhea and in chronic gleet the urine contains small flakes of mucopurulent material which are derived in part from the longitudinal furrows of the mucosa, and in

part from the urethral glands. These are known as *gonorrheal threads* —the Tripperfäden of the Germans. They are yellowish white in color and vary from a few millimeters to a centimeter in length. On microscopic examination they are found to contain pus cells and pavement epithelial cells, in variable number, embedded in a mucinous matrix. They are of special interest from the fact that gonococci may be demonstrated in some of the pus cells of these formations at a time when it is difficult or impossible to obtain any discharge from the urethra directly. If it is desired to ascertain whether there is any involvement of the posterior urethra in a chronic process, the patient is told to flush out the urethra with some of his urine and to hold the balance, while the prostrate is being massaged to empty the glands which are there situated; in the urine which is then voided will be found those threads that are derived from the posterior glands. In these gonococci may be found months and even years after an acute attack.

While the presence of threads is always suspicious, it should be remembered that these formations are not necessarily of gonorrheal origin, but may be met with in individuals who have been addicted to masturbation or sexual excesses in general. They may even then carry bacteria which, however, can be readily distinguished from the gonococcus, being either bacilli or Gram-positive cocci.

The urinary picture in gonococcus septicemia does not differ from that seen in other forms of septicemia (which see).

In females the seat of infection is usually the urethra, the Bartholinian glands, or the cervical canal; from these regions only is it advisable to secure the material for examination.

In the vaginal discharge of adults the organism is only exceptionally found, while in children it is different; the reason for this is probably referable to the differing reaction of the mucosa in the adult female (acid), as compared with the child.

If intracellular diplococci are found either in the male or the female the probabilities are great that the organism in question is the gonococcus. To render assurance doubly strong, however, it is advisable in all doubtful cases to demonstrate that the organism in question is Gram negative; this is especially important in females, where other microörganisms are more apt to enter into consideration. The ultimate proof can only be afforded by cultural methods, but this is rarely necessary.

In the newborn the seat of infection is the conjunctiva, in the purulent discharge from which the organism can be demonstrated.

GOUT

Essential Factors.—Irregular hyperleukocytosis during the attack; increased uric acid content of the blood; tendency to uric acid reten-

tion and diminished elimination; irregular albuminuria and tendency to interstitial nephritis.

The Blood.—*The Red Cells and Hemoglobin.*—These remain unaffected by the gouty process until late in the disease, when in association with other complications a moderate degree of secondary anemia may develop.

The Leukocytes.—During attacks there may be a mild *hyperleukocytosis* (10,000 to 15,000) of the neutrophilic type. Emerson mentions a case in which simultaneously with the rise in temperature and tenderness of the joints the leukocytes rose to 31,000 and fell again as the joint symptoms subsided; this is certainly exceptional. In some cases there is a slight increase of the eosinophiles, which occurs independently of attacks. Occasionally a few neutrophilic myelocytes may be observed.

Neusser, some years ago, described the occurrence of perinuclear basophilic granules which could be demonstrated with Ehrlich's triacid stain, as characteristic of gout and the uric acid diathesis. Futcher and I, independently, have disproved this, and have shown that the same appearances can be obtained in practically any blood specimen. Ehrlich has expressed the belief that the granulesare artefacts.

The Specific Gravity.—The specific gravity of the serum, according to Garrod, is usually between 1.027 and 1.028, rarely under 1.025. When low values are found, these usually find their explanation in complicating conditions (malnutrition, nephritis).

The Alkalinity.—The supposition that in gout a diminished alkalinity exists in the intervals between attacks, and that this increases beyond the normal during the attack has been proved unfounded.

The Uric Acid Content of the Blood.—The uric acid content of the blood is frequently increased (Garrod), but there is no relation between the amount and the occurrence of the paroxysms. Magnus-Levy found 50 to 70 mg. (normal, 3 to 6 mg.) per liter as average quantities. This condition, however, is not pathognomonic of gout, as a similar increase may be noted in other conditions which have no connection with gout.

In several cases Garrod also found an increased content of *oxalic acid* (sc. its calcium salts).

Freezing Point of the Blood.—A study of the *molecular concentration* in a small number of cases has shown a marked lowering of the freezing point of the blood (—0.76° and —0.82° C.) during the attack, while in the interval normal values were obtained.

The Urine.—The general examination of the urine in gout shows little of interest. At the onset of the attack the *quantity* is diminished, the *specific gravity* high and on standing a *deposit of urates* is apt to develop; toward the end of the attack the flow increases and the specific gravity falls. In the interval the amount is normal, unless gouty nephritis has developed, when polyuria will be observed.

A study of the *nitrogen partition* has not shown any points of special interest; the urea and ammonia fractions are normal both during the attack and in the interval; exceptionally the ammonia values are a little higher than normal during the attack. The *acidity curve* shows no material deviation from the normal. A study of the *inorganic salts* and notably of the ratio of the *phosphoric acid to the total nitrogen* also has revealed no points of special interest. This centres altogether in the elimination of *uric acid* and the *purin bases*. In the past it was thought that on an average the gouty patient eliminates less uric acid than the normal individual, and that this decrease occurs especially during and immediately preceding the attack, while afterward it increases again to approximately normal values (Garrod). In a general way this view is still held, but it must be borne in mind, nevertheless, that the diagnosis gout can scarcely be made in the laboratory upon this basis; the average daily output in the gouty individual is after all the same as in the normal control person. The ratio between purin bases and uric acid, which, according to Kolisch was thought to be altered in a manner characteristic of gout, has likewise been shown to differ in no way from what is found in the non-gouty individual, Kolisch's results being obtained with a method which has since been shown to yield too high purin values. Of greater interest are the data which have been obtained in gouty patients on a diet free from purins, as compared with a diet containing purins, where accordingly the exogenous portion of the uric acid output is the variable quantity. In the former case the values in the interval are generally about normal, standing nearer to the lower than the upper limit, however. So far as the results on a diet of known purin-yields are concerned, the following has been ascertained: (1) There are times of normal and of defective elimination of uric acid which occur spontaneously. (2) During periods of frequent attacks the elimination is markedly delayed and diminished. (3) The introduction of small amounts of purin may be followed by normal elimination, while the response to larger quantities may be deficient. (4) Long-continued administration of a diet poor in purins seems to increase the power of elimination of uric acid (v. Noorden). The following example will furnish an idea of the uric acid curve in a concrete case (Brugsch), the patient being on a diet free from purins.

Attack	0.708 and 0.675 gram
Interval of four days	0.376 (average)
Attack	0.618 (first day)
Subsequent days of attack	0.375 (average of eight days)
Light attack	0.585 (average of two days)

Glucosuria is not a feature of gout, but may be observed as digestive type (following the ingestion of 100 grams of glucose) in alcoholic cases or cases complicated by obesity. Actual diabetes is

only observed in a very small percentage of cases. The older statement that the two conditions could alternate has been disproved (v. Noorden).

"Functional" *albuminuria* of mild grade is frequently observed at the onset of an attack, but disappears in the interval. When it persists there is ground for suspicion that definite changes in the kidneys have developed. Interstitial nephritis is, as a matter of fact, a frequent complication of gout and especially common as a terminal factor.

According to Magnus-Levy and v. Noorden the *urobilin* content of the urine is frequently increased during the attack.

HEART DISEASE (CHRONIC VALVULAR)

Essential Factors.—Irregular polycythemia and hyperleukocytosis; presence of "Herzfehlerzellen" and blood in the sputum; occurrence of transudates; tendency to moderate albuminuria and cylindruria.

The Blood.—*The Red Cells and Hemoglobin.*—In uncomplicated cases of chronic valvular disease, so long as compensation is adequate, the blood shows no abnormalities. When this fails anemia is apt to develop, which may be obscured, however, by a capillary polycythemia (reaching 6,000,000 or more). The tendency to anemia is said to be more marked in aortic than in mitral cases, but according to Cabot mitral disease also may be associated with severe anemia. In 20 of his series of 91 cases the average count was 3,400,000 and in 3 of these nucleated red cells (normoblasts) were found.

The Leukocytes.—In well-compensated cases the leukocyte count is normal. In patients, however, who are sufficiently inconvenienced as to seek medical advice, the number is often increased, even though no complicating conditions exist which in themselves would give rise to hyperleukocytosis. In Cabot's series of mitral cases the white count exceeded 11,000 in 51, and 16,000 in 32; some of these cases, however, were complicated by pulmonary infarction or nephritis which may have influenced the counts. The highest figures are usually noted as death approaches. Of his 27 aortic cases, 10 gave values exceeding 11,000, the highest count being 31,000. The differential count sometimes gives normal values, showing that the hyperleukocytosis is not real, but referable to capillary stasis; otherwise it is of the neutrophilic type. The eosinophiles remain unaffected in some cases, while in others they are diminished.

General Characteristics.—During the stage of serous plethora which is seen when compensation fails acutely there is a drop in the specific gravity and the albumins, owing to dilution of the blood with lymph from the tissues, while in the stage of chronic stasis with cyanosis there is a corresponding concentration of the blood and hence an increase of the specific gravity and the albuminous content.

The Sputum.—In all forms of chronic valvular disease, but especially in the mitral forms, chronic bronchitis is a common complication. The sputum is usually mucoid in character with relatively little tendency to become purulent; when stasis is extensive it may become serous and quite abundant. Bleeding is common; usually the sputum is merely tinged with blood, but at times the hemorrhage may be profuse. When bloody expectoration begins rather abruptly in cases of mitral stenosis, the inference is usually justifiable that hemorrhagic pulmonary infarction has occurred. The sputum then either consists of pure blood or of blood and mucus, mixed in variable proportion, and is but little frothy. In other cases the sputum looks pneumonic, and in still others there may be none. In cases of long-continued passive congestion, especially when associated with mitral disease, and here more particularly with stenosis, the mucoid sputum is frequently streaked or dotted with rust-colored pigment, which on microscopic examination is found to be inclosed in alveolar epithelial cells and leukocytes—the Herzfehlerzellen of the Germans. The continuous presence of these in large numbers is sometimes of diagnostic significance.

Transudates.—The formation of transudates in the pleural, pericardial, and peritoneal cavities is a common complication in valvular disease, when compensation has once been seriously broken. It is frequent in mitral cases, while in aortic cases it rarely occurs until a relative mitral insufficiency has been established. The quantity which may be obtained at one time is, of course, variable, but usually amounts to several liters. The fluid is clear and of a pale yellowish color with a greenish fluorescence. The specific gravity, as compared with inflammatory effusions, is low, rarely exceeding 1.015; in peritoneal transudates it may be as low as 1.005. The albuminous content varies between 1 and 2.5 per cent., the largest amount being found in effusions of pleural origin. In contradistinction to the inflammatory effusions the transudates do not coagulate spontaneously unless blood is accidentally present. Too much reliance should not be placed upon this point, however, as exudates likewise do not always coagulate, and as clotting of transudates in the presence of blood may take place within the body.

Chemical examination frequently reveals the presence of urobilin, even though red corpuscles and blood coloring matter in solution be absent. Nucleo-albumin is not found, while a mucoid substance can be demonstrated in fair amounts; in exudates this is present only in small quantity.

The Urine.—So long as a valvular lesion is well compensated the urine presents no abnormalities. With failure of compensation, however, the quantity diminishes very notably, the color is darker, the specific gravity higher, and the acidity greater; sediments of uric acid or amorphous sodium urate are common. If venous stasis

is at all considerable albuminuria develops; the quantity varies, but rarely exceeds 0.1 to 0.2 per cent., unless the disease has advanced to a stage where distinct anatomical changes have resulted. Microscopic examination reveals the presence of a few hyaline casts, red corpuscles, and leukocytes.

In extreme cases the renal insufficiency as the result of stasis may become so extensive that the secretion of urine is arrested almost entirely and the patient dies in uremic coma. Actual nephritis, whether acute or chronic, may also develop, with corresponding urinary changes (which see). Renal embolism may be suspected, when marked hematuria occurs.

HEART DISEASE (CONGENITAL)

Essential Factors.—Absolute polycythemia with high hemoglobin value and hyperleukocytosis.

The Blood.—*The Red Cells and Hemoglobin.*—In all cases of congenital heart disease in which chronic cyanosis is a feature, polycythemia is almost invariably observed. In Townsend's series of 14 cases the counts ranged between 5,600,000 and 11,800,000, and in another series of 13 cases collected by Vierordt, between 6,700,000 and 9,600,000. The hemoglobin value is usually correspondingly high. Banhalzer has reported a case in which this amounted to 160 per cent., and Moritz gives fluctuations between 150 and 170.

The Leukocytes.—The leukocytes are also increased, but not proportionately so, the figures varying between 8800 and 16,000. Differential counts are unfortunately not available.

The Specific Gravity.—The specific gravity is high (1.071 to 1.081).

The Sputum.—The sputum frequently shows admixture of blood, and at times free hemorrhages may be observed.

The Urine.—The urinary picture is essentially the same as that noted in chronic valvular disease in adult life (which see).

HEMOPHILIA

Essential Factors.—Irregular anemia; diminution in the number of the plaques; delayed coagulation.

The Blood.—*The Red Cells and Hemoglobin.*—Unless recent hemorrhages have taken place, the red count and hemoglobin values may be perfectly normal. Otherwise the degree of anemia is proportionate to the amount of blood that is lost and to the frequency of the attacks. Blood regeneration, however, is remarkably rapid, more so, in fact, than in non-hemophilic individuals.

Leukocytes.—Regarding the leukocytes, there are no available data on which to base a proper account. So far as my investigations have gone the number is practically unaffected; occasionally there is a tendency to minimal normal values.

The Plaques.—In contradistinction to the purpuras, where the plaques are diminished, hemophilics show no deviation in their number.

The Coagulability.—Coagulation of the blood is either greatly retarded or does not occur at all, so that fatal hemorrhages may follow the infliction of a trifling wound. With Howell's method a coagulation time of 90 to 300 minutes may be expected.

HEPATITIS SUPPURATIVA

(Multiple liver abscess)

Essential Factors.—Secondary anemia; hyperleukocytosis with septic factor; bacteriuria; demonstration of pus by exploratory puncture; right pleural effusion; rupture into the lung, the bowel, or the pelvis of the right kidney.

The Blood.—*The Red Cells and Hemoglogin.*—The blood picture, in so far as the question of anemia is concerned, depends to some extent upon the underlying cause of the suppurative hepatitis. When this is a secondary manifestation of a general pyemia the patient may have already developed a marked grade of anemia before the beginning of the hepatitis. In those cases, on the other hand, where liver abscess develops from gallstones the patient may present a normal count and normal hemoglobin value at the onset. Subsequently, however, there may be a considerable degree of anemia which is attributed directly to the abscess.

The Leukocytes.—The leukocyte count is probably increased in every case of suppurative hepatitis of bacterial origin, during the active stage of the disease, though the degree of increase as in all septic conditions may be quite variable, and periods of hyperleukocytosis may alternate with such of relatively normal counts. Here, as in other septic states, the differential count is more valuable than the absolute count. It shows the typical septic factor not only at a time when the absolute count is increased beyond the maximal normal limit, but frequently even when this is normal. The absolute counts which are mentioned by several writers are not applicable at this place, as there has been no separation of the bacterial from the non-bacterial (amebic) cases. I have no doubt that the average figure would be materially higher if the latter were eliminated. In several cases in which abscess formation developed as the result of a pylephlebitis I have seen counts of 50,000. In the chronic stage of the disease, and this has reference almost exclusively to the gallstone abscesses, the absolute count may show but little or no deviation from the normal, but even then, as I have just indicated, the differential count may indicate the existence of a septic process. When the abscess is well encapsulated or has perforated to the outside,

into the bowel, a bronchus, or the pelvis of the right kidney, the leukocytic picture may again become normal.

The Bacteriology.—The bacteriological findings depend upon the causative factor, and are essentially those of a generalized septicemia.

The Pus.—The pus which may at times be obtained by exploration of the liver with the aspirating needle often has a reddish meat color which is fairly characteristic. Microscopic examination reveals a predominance of polynuclear neutrophilic leukocytes, possibly threads of fibrous tissue, more rarely well-preserved liver cells, a large amount of detritus, and here and there some of the offending bacteria, among which the Staphylococcus aureus, streptococci, and the colon bacillus are the most common. More rarely the B. pyogenes fœtidus, B. typhosus, B. dysenteriæ, B. pyocyaneus, the pneumococcus, Proteus vulgaris, and the Actinomyces bovis are found.

Pleural Effusion.—One feature to which special attention must be called in the diagnosis of these cases is the occasional presence of fluid in the right pleural cavity. This is usually moderate in amount (100 c.c. or thereabout), and, like the peritoneal fluid in suppurative appendicitis, more or less turbid from the presence of leukocytes. Its absence, however, has no diagnostic significance in a negative sense.

Sputum.—When perforation of a liver abscess takes place into a bronchus the diagnosis may be made from the macroscopic appearance of the sputum. (See Abscess of the Lung.)

The Urine.—This shows no features which can be regarded as in any sense peculiar to the disease in question, unless it be the occurrence of pyuria in the rare cases where the abscess perforates into the pelvis of the right kidney. (See Pyelitis.) In other respects the features are those of a more or less acute febrile process (see Septicemia), with a mild grade of choluria in some.

(For a consideration of the laboratory findings in amebic liver abscess see Amebiasis.)

HERPES ZOSTER

(Herpetic fever)

The Blood.—According to Sabrazès and Mathias there is a leukocytic increase at the beginning of the eruption which reaches its height by the third day; after this there is a decline to the normal and a subsequent rise during the period of desiccation and desquamation. The leukocytosis is of the neutrophilic type with persistence of the eosinophiles and even a mild hypereosinophilia. Myelocytes may be present in small numbers.

The Urine.—At the onset of the disease there is moderate oliguria (900 c.c. average); the specific gravity is normal or slightly elevated;

the reaction strongly acid. Albuminuria of slight degree is seen in approximately one-fourth of the cases, at the height of the disease, while after defervescence it is very rare. Sugar is absent.

HISTOPLASMOSIS

The Blood.—Darling has reported observations on 3 cases. Detailed blood examinations are unfortunately lacking. In one the hemoglobin percentage was 60 and in another 70. The only leukocyte count recorded was 2200.

The diagnosis of the disease was only established post mortem, but it would seem from the similar findings in Kala-azar that in suspected cases splenic puncture and examination of corresponding smears might lead to a correct diagnosis during life.

HODGKIN'S DISEASE

Essential Factors.—Relatively late development of anemia, which is usually of a moderately chlorotic type; absence of hyperleukocytosis, with relatively normal leukocytic formula tending to lymphocytosis.

The Blood.—*The Red Cells and Hemoglobin.*—In the slowly developing cases the red count and hemoglobin values are frequently normal, or the latter is slightly diminished, when the patient first comes under observation. In the more rapidly progressing cases the anemia sets in earlier and is more marked. In all cases, however, anemia is a feature of the disease, when this is at its height. This is usually of the chlorotic type with lowered color index, but occasionally the loss of red cells is more extensive, equalling what is seen in pernicious anemia, and may then also be associated with a high color index; this, however, is rare. The average number in Da Costa's series was 3,591,423, the lowest, 1,300,000, and the highest, 5,225,000, with more than one-third of the cases having 4,000,000 cells or more. The average hemoglobin value was 55.3, the lowest, 30, and the highest, 81 per cent.

Morphological examination shows no material changes, unless the anemia is marked, when those deviations from the normal which are common in secondary anemias may be observed. Nucleated red cells are scarce; megaloblasts are only seen in extreme cases, and are always outnumbered by the normoblasts.

The Leukocytes.—In cases of a year or less in duration the total leukocyte count is normal or slightly increased, with a normal or decreased percentage of neutrophiles. In cases of longer duration there is apt to be a sharp rise in the total number (up to 100,000) due to an increase of the neutrophiles. The eosinophiles are usually diminished when the neutrophiles are increased and are frequently absent at this stage of the disease, while normal values are usual

when the neutrophiles are not increased. Bunting lays a good deal of stress in diagnosis upon an increase of the old forms of the large mononuclears—the so-called transition forms (8.6 to 15 per cent. during the first stage of the disease). The lymphocytes may show a moderate increase early in the disease, but gradually their number drops as the neutrophiles go up.

The Plaques.—The plaques are increased and often run together in large masses.

The alkalinity and *specific gravity* are diminished in the decidedly anemic cases, and in these the *coagulation time* also is frequently much increased.

The Urine.—The urine shows no special abnormalities which could in any way be regarded as characteristic. Jolles reports that he found the uric acid and xanthin bases very much increased, although there was no hyperleukocytosis nor evidence of an increased leukocytolysis. The same writer and Stein claim to have found nucleohiston. Albuminuria may be observed *sub finem vitæ*, or during febrile paroxysms, and in rare cases transitory glucosuria has been observed.

HYDATID DISEASE

Essential Factors.—Irregular anemia; hyperleukocytosis with hypereosinophilia; presence of succinic acid, sodium chloride and the component elements of the cyst in the cystic contents; presence of the same in the sputum when lung involvement has taken place; the same in the urine in renal cases.

The Blood.—*The Red Cells and Hemoglobin.*—In uncomplicated cases there is little or no anemia, while associated suppurative processes may lead to a marked loss of red cells and hemoglobin. In one instance (hydatid of the liver), reported by Seligman and Dudgeon, the red count before operation was 6,290,000, while two months and a half later, owing to suppuration, no doubt, only 2,934,000 were counted.

The Leukocytes.—The leukocytes are usually, but not invariably, increased. High values are more apt to be seen in suppurative cases. Longridge reports an instance of this kind with 18,000 and Cabot one with 34,000. The differential count in most cases shows a variable degree of eosinophilia at the expense of the neutrophiles; usually this is moderate, amounting to 10 to 20 per cent., but at times much higher values (57 per cent.) are encountered. Not all cases, however, are associated with hypereosinophilia. Dr. J. Ramsey of Launceston, Tasmania, has kindly sent me his findings in 5 cases. In 4 of these there was no suppuration, but in spite of this, increased eosinophile values were found in only one (28.4 per cent.); the others presented normal values. In one suppurating case they were absent. That suppurative cases do not necessarily show absence of eosinophiles,

however, is proved by a case of Longridge's, in which 1.4 per cent. were counted. After operation the leukocytic formula returns to normal.

The Cystic Contents.—The normal fluid in hydatid cysts is clear like water, neutral (sometimes faintly acid or alkaline), of a specific gravity of 1.000 to 1.015, and rich in sodium chloride. By transmitted light it is faintly opalescent. It contains no albumin, or only a trace. Succinic acid and sugar may be present in small amount. A sediment, if present, is chiefly composed of scolices, debris of parenchyma, calcareous particles, and hooklets. Hematoidin crystals may be found if blood has entered the cyst. When tapping has been done, albumin may subsequently be found in greater quantity; the same is the case in degenerating or suppurating cases. With the death of the hydatid the fluid changes greatly in character. It becomes more turbid, fatty globules may be found with granular cells and crystals of cholesterin. In long-standing cases the content may become of putty-like consistence, in which remains of the gelatinous membranes may be demonstrated by floating the material out in water. If calcification has taken place, hooklets may still be found by rubbing up the material with water in a mortar. When suppuration takes place, neutrophilic leukocytes are first found between the cyst and its adventitious capsule; ultimately the cyst may soften down and burst, membranes, scolices, and hooklets floating about in the pus.

(For a description of the cysts and parasites see the section on Parasitology of the Sputum.)

The Sputum.—When a hydatid cyst of the lung, liver, or neighboring organs has ruptured into the larger or smaller divisions of the bronchi, quantities of clear, watery fluid giving the characteristic tests for hydatid fluid (succinic acid and sodium chloride) may be coughed up and be found to contain perhaps (1) small cysts full of clear fluid, from the size of a pin's head upward—the daughter or granddaughter cysts; (2) whitish, dot-like bodies just visible to the naked eye, when single, or more evident when grouped in colonies—the scolices or echinococcus heads; (3) some of the component parts of the cysts or scolices, such as collapsed cysts, resembling grape skins, or pieces of the gelatinous membrane of a mother or daughter cyst, or hooklets and calcareous particles from the bodies of the scolices, which are visible only under the microscope (Ramsey)

When the hydatid has suppurated before rupture, pus in large or small amounts takes the place of the clear fluid, or is mixed with it.

Sputa may be expectorated from a hydatid cavity of the lung for months or years, and are then usually of a purulent or mucopurulent character and perhaps blood tinged. On examination with a low power a thick smear may reveal pieces of laminated membrane or hooklets.

When a hydatid of the liver has ruptured into a bronchus the sputa may be bile-stained.

The Urine.—When a hydatid of the kidney has ruptured into the urinary tract the findings in the urine will be essentially the same as those just described; blood may also be present in variable amount. At times the presence of a cyst may lead to pyelitis and in rare cases to gangrene.

HYSTERIA

The laboratory findings in hysteria are essentially the same as those described in the section on neurasthenia. Aside from these one should be prepared for surprises of almost any kind. Hair, teeth, fish-bones, wood, etc., and even snakes and frogs may be shown the physician as having been passed in the urine. I had occasion to examine some gravel "that had been passed from time to time by an hysterical patient in large amount; every attack being accompanied by agonizing pains shooting down into the lower abdomen." The gravel on examination proved to be mortar from the cellar walls of the patient's house.

A few years ago Dr. Watson, of Baltimore, brought me material which the patient expectorated with great difficulty. On examination this was found to represent raw chicken lungs, and when turkeys were provided for the family household in the place of chickens, turkey lungs appeared from the patient's interior.

In another instance a little girl, aged twelve years, put red paint from her paint box into her urine in order to attract attention and to escape going to school.

In still another case a young girl maintained an inflammation of a lower eyelid by various means, and caused a copious discharge by the application of vaselin.

INFLUENZA

Essential Factors.—Absence of notable hyperleukocytosis; absence of septic factor; tendency to lymphocytosis; presence of the influenza bacillus in the sputum.

The Blood.—*The Red Cells and Hemoglobin.*—These are not materially affected by an ordinary attack of influenza, but in the severer cases a moderate anemia of the chlorotic type may result.

Several investigators claim to have isolated the *influenza bacillus* from the blood, while others, and among these Pfeiffer himself, obtained negative results. Jehle states that he found them in 22 of 48 cases of scarlatina and also in a considerable number of cases of measles, varicella, and whooping cough. His results, however, lack confirmation.

The Leukocytes.—Series of cases in which the diagnosis influenza was established by bacteriological methods and in which adequate

blood examinations were made are unfortunately lacking, and in judging the findings of various investigators who have reported their blood counts, we have no means of ascertaining whether the cases were really all infections with Pfeiffer's bacillus, or whether the list does not include other winter infections as well. This uncertainty no doubt is responsible for the varying results which have been reached by different observers. From the available data it appears, however, that absence of hyperleukocytosis is the rule, but that in some cases the number may be increased. In Cabot's series of 309 cases values exceeding 10,000 were obtained in 135 cases. As I have said, however, we have no means of ascertaining whether all or any of these 135 cases were pure infections with the influenza bacillus. I have no records of many absolute counts myself, but my impression has been that it is unusual to find counts above 12,000, and that in most cases the number is not increased. Even in those cases in which an influenza pneumonia develops the number of the leukocytes is but little or not at all affected. While the absence of a definite hyperleukocytosis may thus be an important factor in the diagnosis of obscure cases, I am personally inclined to attach more significance to the results of the differential count. My experience has been that cases of true influenza (controlled by bacteriological examination) show a distinct lymphocytosis and absence of the septic factor. I would emphasize the importance of these findings in the diagnosis of the disease and especially in differentiating it from infections with the pneumococcus. The increase of the mononuclear elements is practically confined to the lymphocytes and usually amounts to from 30 to 40 per cent.; but it is not uncommon to meet with much higher values. The eosinophiles are diminished or absent during the active stage of the disease (unless complicating factors, such as a gonococcus infection, are at the same time active, which in themselves would produce a hypereosinophilia). With convalescence they return to normal again, while the lymphocytosis usually persists for a number of days or even longer. Emerson states that of the Hopkins cases, nearly all, in which several counts were made, showed early a very low count, then a sharp rise, which fell after the temperature came to normal. He concludes that from the diagnostic standpoint the leukocyte curve is of value, and not a single count. It would follow also that the absolute count is of value only early in the disease.

The Sputum.—As the bacteriological diagnosis of influenza must be based upon the demonstration of the corresponding organism in the sputum, it follows that the diagnosis of the disease, strictly speaking, is only possible when the patient has sputum. Nevertheless, there can be but little doubt that many cases of true influenza occur in which the involvement of the respiratory tract is insignificant and in which no sputum can be obtained. In such cases the clinical history and the condition of the blood must decide the diagnosis.

The appearance of the sputum in the respiratory form of the disease will vary somewhat with the extent to which the air passages are involved, but is not very characteristic. The specific organism is found both free and inclosed in leukocytes; it is often present in enormous numbers, and may at times be obtained in pure culture. It is important to note that its occurrence is not confined to periods of epidemics, but that it may persist in the air passages for months and even years following the primary infection, and may be responsible for many cases of chronic bronchitis. It may, moreover, become a secondary infecting agent, and has thus been found in various cases of whooping cough, tuberculosis, etc. Lord found the organism in 60 cases out of 100 non-tubercular coughs, and of these in almost pure culture in 29. In one instance there was reason to believe that the infection had existed for forty-four years.

The Urine.—The urine shows nothing characteristic; it merely presents the general features of a febrile attack. Albuminuria is not present in cases of moderate severity, but may be found in the severer forms, accompanied by a few hyaline casts.

INSOLATION

(Sunstroke; thermic fever)

Essential Factors.—Initial polycythemia and hyperleukocytosis.

The Blood.—*The Red Cells and Hemoglobin.*—Owing to the occurrence of blood concentration early in the attack there is frequently an abnormally high red count with correspondingly high hemoglobin value, which at this time is very apt to obscure the coincident hemolysis, that takes place in some of the cases.

The Leukocytes.—In thermic fever a high leukocyte count is apparently the rule, but there is considerable irregularity in the time and duration of the rise. Lewis and Packard report that in some of their cases a leukocytosis of 12,000 to 13,000 was noted on admission. In most of the cases in which there was a primary rise this was followed by a fall and then a second increase in their number.

In Cabot's series of 15 cases the count ranged from 8200 to 24,000. Differential counts are unfortunately not available; they would, no doubt, show whether the hyperleukocytosis in question is the result of blood concentration, or the outcome of a direct stimulation of the blood-forming organs.

In some instances in which marked hemolysis has taken place pigment-bearing leukocytes may at times be encountered.

In simple *heat exhaustion* hyperleukocytosis may also be observed. Cabot's figures range between 4450 and 24,000, one-third of the cases showing values higher than 10,000.

The Urine.—The urine may contain a considerable amount of albumin with hyaline and granular casts, and in some cases hemoglobinuria has been observed. Toward the end there may be anuria.

INTESTINAL HELMINTHIASIS

Essential Factors.—Anemia with high color index in bothriocephalus infections; anemia with low color index in hookworm and roundworm infections; hypereosinophilia; presence of the corresponding parasites, their embryos or eggs in the feces; presence of Charcot-Leyden crystals in the feces; occult or manifest bleeding in strongyloides infections.

The Blood.—*The Red Cells and Hemoglobin.*—While a survey of the literature shows that all forms of intestinal parasites *may* cause anemia, certain ones are more apt to do so than others. This is especially true of the broad tapeworm (Bothriocephalus latus), the hookworm (Uncinaria duodenalis), and the roundworm (Ascaris lumbricoides), while the ordinary tapeworms (Tænia solium and saginata) and the common seatworm (Oxyuris vermicularis) are relatively harmless; the whipworm (Trichocephalus dispar) likewise is usually innocuous, but may at times become dangerous. Strongyloides intestinalis may cause anemia in consequence of the long-continued diarrhea and almost continuous intestinal bleeding to which it gives rise, but it is sometimes obscured by a relative polycythemia.

Bothriocephalus anemia in many respects resembles the ordinary cryptogenetic type of pernicious anemia. Here as there the oligocythemia is very extensive and exceeds the oligochromemia, the color index accordingly being increased. Here as there poikilocytosis, anisocytosis, and granular degeneration of the red cells are marked. The megaloblasts outnumber the normoblasts in over half of the cases. The diagnosis between the two conditions can, indeed, be made only by the demonstration of the offending parasite and the recovery of the patient which follows its removal. In Schaumann's series of 38 cases in which blood examinations had been made the average red count was 1,292,000, and in only one instance was the initial count lower than 2,500,000; in 28 of the cases it did not exceed 1,500,000, and in one case it was under 500,000. In all the cases the hemoglobin was lower than 45 per cent. and in 31 it did not exceed 30. The color index was higher than 1 in 30 cases, the average being 1.09. The *plaques* were diminished.

In *hookworm anemia* the loss of red cells may be as extensive as in pernicious anemia, but, on the other hand, there are numerous cases of mild infection in which no anemia whatever is noted. In definitely anemic cases the extent of the oligocythemia seems to vary with different epidemics, but it is probably dependent to a great extent upon

the number of parasites. In Boycott and Haldane's series the figures varied between 1,533,000 and 5,384,000, and in that of Ashford between 687,776 and 3,084,440. In individual cases there may be remarkable fluctuations. In a case reported by Yates the count dropped from 2,500,000 to 800,000 within a week, and in one mentioned by Capps, from 2,576,000 to 748,000 in two months.

The loss in hemoglobin exceeds the oligocythemia in nearly all cases, so that, in contradistinction to pernicious anemia, the color index is low. In Boycott and Haldane's series the hemoglobin values varied between 17 and 104 per cent., and in Ashford's between 14 and 30 per cent., which in itself illustrates the difference in different epidemics, Haldane's cases occurring among Cornish miners and Ashford's being inhabitants of Porto Rico. Sandwith's average index of 173 Egyptian cases was 0.54.

The morphological changes in hookworm anemia depend upon the severity of the case; in mild cases there are none, while in severe cases the picture may closely resemble that of pernicious anemia. The pallor of the red cells, however, is a distinguishing feature. Whether or not stiple cells occur in increased numbers I have been unable to ascertain. Erythroblasts are common in the markedly anemic cases; they are usually normoblasts; megaloblasts may also be present, but they are always in the minority.

Regarding the frequency and extent of *roundworm anemia* the literature is rather barren. That the worm is capable of causing anemia can hardly be doubted, and is evidenced by the fact that it disappears after the expulsion of the parasites. A case of this kind is cited by Huber, where the red count rose from 2,450,000 to 4,200,000 within two weeks after the passage of large masses of roundworms. The deciding factor in such cases is undoubtedly the number of the worms; in the case just cited this was estimated at from 200 to 300.

Trichocephalus dispar is apparently not often the cause of marked anemia, but that it may be so is suggested by the reports of several observers. It may indeed assume a pernicious character; but, as in Bothriocephalus anemia, the color index is diminished, whereas in the Biermer type it is increased. In one of the reported cases the red count had fallen to 600,000.

In *strongyloides infections* red counts and hemoglobin estimations have only been reported in a few cases. Brown gives a count of 3,882,000, with 65 per cent. of hemoglobin, and Emerson, two of 5,420,000, with 82 per cent. of hemoglobin, and of 3,560,000, with 57 per cent., respectively. The lowest figure which I have been able to find was 760,000.

The common *tæniæ*, as I have already mentioned, rarely give rise to notable anemia.

The Leukocytes.—In the intestinal helminthiases the total number of the leukocytes is not usually increased at a time when the presence

of the parasite is first discovered. Whether hyperleukocytosis occurs earlier in the course of the infection is impossible to say, but seems not improbable in view of Boycott and Haldane's observations among Cornish miners afflicted with hookworm disease. Here it was noted that hyperleukocytosis, sometimes of high grade (20,000 to 56,000), occurred among those patients in whom the infection was of recent date and the symptoms mild, while the lowest counts (3800 to 6800) were obtained where the disease had existed for several years. Ashford's uniform normocytosis, excepting in two cases otherwise accounted for, is probably best explained on this basis. Where marked anemia exists leukopenia may occur; this is common in bothriocephalus cases.

One of the most interesting features of the blood picture in the intestinal helminthiases is the common occurrence of hypereosinophilia. This is particularly marked in hookworm infections, where it is present at some time in the course of the disease in practically every case. It is most marked in early cases and in those late cases where blood regeneration is still active; a count of from 20 to 50 per cent. is then not uncommon. In chronic cases or in those who have been profoundly anemic for a long time the count is more apt to be low than high. When there is a fall of eosinophiles unaccompanied by improvement in the patient's general condition, death is apt to follow (Ashford). High values are also commonly observed in Schistosomiasis japonica, where the eosinophilia may amount to 60 per cent.

In the other intestinal parasitic diseases hypereosinophilia is not so constant and rarely so high. In bothriocephalus infections an increase is, in fact, unusual. Schaumann states that in 25 of his cases the eosinophiles were very scanty, and in one case only somewhat increased. This has been the experience of others also, at least in those cases in which active and pronounced anemia existed. After removal of the worm, however, the eosinophiles may temporarily increase beyond the normal (9 per cent., in a case reported by Gilman Thompson).

The ordinary tapeworms (T. solium and saginata) can unquestionably give rise to hypereosinophilia, as is evidenced by a case of Leichtenstern's in which 34 per cent. were counted. In many instances, however, normal values are met with at the time when the discovery of the infection is first made. Early infections may possibly be more frequently associated with increased values. In one case of Tænia nana Buckler found 7 per cent.

The same holds good for the ascarides and oxyurides. Buckler gives a count of 16 per cent. in an infection with the latter and of 19 per cent. in the case of the former. He cites a case, on the other hand, where, in spite of a mixed infection with oxyuris, ascaris, and Tænia saginata the eosinophile count showed only 5.7 per cent.

In the few cases of strongyloides infection of which I could find records, a moderate eosinophilia existed (6.3 to 13.5 per cent.). In one case of my own observation the count was 23 per cent.

In the trichocephalus cases the eosinophiles rarely fall below 5 per cent. One case of Brown's suggests that eosinophilia does not necessarily occur, for in spite of a mixed infection with strongyloides, trichocephalus, and uncinaria, there were only 5 per cent. of eosinophiles. In a case reported by Cecil and Bulkley, on the other hand, the eosinophile percentage was 44.

In some of the cases of intestinal parasitic infection the small mononuclears are increased and the polynuclear neutrophiles diminished, even though the eosinophiles show no material deviation from the normal. This, however, is inconstant, and I note that in Boycott and Haldane's, as well as Ashford's series of hookworm cases the small mononuclears were not increased, while the decrease of the polynuclear neutrophiles was proportionate to the increase of the eosinophiles.

The mast cells are not affected.

The Gastric Juice.—Regarding the condition of the gastric juice there are no available data, excepting in bothriocephalus cases, where, according to Schaumann, it was "usually feebly acid;" in one case free hydrochloric acid could be demonstrated.

The Feces.—The diagnosis of the various intestinal helminthiases must be based upon a careful examination of the feces. The presence of eosinophilia, in the absence of other readily apparent causes which can produce the same, should always excite suspicion, but the ultimate diagnosis requires the demonstration either of the offending parasite or its ova. This is usually a simple matter. Frequently administration of a brisk cathartic preceded by a little santonin or male fern is sufficient to bring away a roundworm, or a few proglottides, in the case of the common tapeworms. If not, a search is made for ova or larvæ. Ova may be obtained in the case of the Uncinaria, Bothriocephalus latus, Tænia solium, saginata, and nana, Trichocephalus dispar, Ascaris lumbricoides and Schistosoma japonicum. Oxyuris usually does not lay its eggs until after the discharge of the feces, but occasionally they may be found. In the case of the strongyloides free larvæ appear in the feces, while the eggs are rarely (if ever) found. The number of eggs and larvæ which appear in the feces differs, of course, in different cases, depending in turn upon the number of adult worms or in the case of the tæniæ upon the number of ripe segments. Where this is small the number of eggs may be small. In other cases where the feces contain large numbers it will not be surprising to find many adult parasites. The hookworms are often especially numerous, and at autopsy of severe cases the jejunal mucous membrane may be found literally studded with them; in such cases a bit of fecal matter stirred up in water may

be seen to contain half a dozen eggs or more to every field of the low power. At other times it may be necessary to hunt over several slides before one is found. The trichocephalus also may give rise to many eggs. In one case reported by Moosbrugger 10,400 were found in 1 c.c. of fecal contents, corresponding to a total daily elimination of approximately 5,000,000 eggs a day, from which the number of female worms in the intestine was estimated as between 1000 and 2000. Proglottides of tapeworms are sometimes discharged without a stool and may be very numerous. One of Küchenmeister's patients passed 1200 segments in eighty days, corresponding to a length of 33 meters (Huber). The number of ascarides which inhabit the intestinal tract at one time does not exceed a dozen, as a rule, but at times they may be very numerous. Volz mentions the passage of 808 worms in seventeen days, and Pole cites another in which 441 were passed within thirty-four days.

The general condition of the feces will depend upon the question, whether or not the offending parasites give rise to inflammatory changes in the intestinal mucosa. In strongyloides infections where this occurs to a marked extent diarrhea with the passage of blood is a common symptom, and is apt to persist in spite of treatment. In bothriocephalus infections diarrhea occurs in fully one-half of the cases, while the other tapeworms (T. solium, saginata, and nana) usually do not give rise to intestinal symptoms. The same is true of the seatworm and the ascarides. The latter, on the contrary, may give rise to occlusion of the bowel by becoming massed together. Considering the size of these worms and the large number which an individual may harbor at one time, this does not seem surprising. In trichocephalus infections catarrhal diarrhea only occurs when the worm is present in great numbers, and as this is the exception it usually does not give rise to symptoms. At times, however, the patient may be very ill with bloody diarrhea.

The Urine.—The urine shows no changes which could be attributed to the presence of the parasites.

INTESTINAL OBSTRUCTION

Essential Factors.—Hyperleukocytosis of the neutrophilic type, with decrease or absence of the eosinophiles; "fecal" vomiting; oliguria; increase of indican in obstruction of the small intestine; irregular albuminuria and cylindruria.

The Blood.—*The Red Cells and Hemoglobin.*—The blood picture in intestinal obstruction, so far as the red cells and hemoglobin are concerned, depends upon the underlying pathological condition and requires no special discussion at this place.

The Leukocytes.—In intestinal obstruction an increase in the number of the leukocytes is one of the most constant symptoms.

While ordinarily of moderate intensity, unusually high counts, viz., 50,000 or more, are observed in isolated cases. Bloodgood states that in a large group of cases the leukocyte count may rise to 20,000 within twelve hours after the beginning of the obstruction. Within the first twelve to twenty-four hours a few observations would demonstrate that if the leukocyte count rises above 25,000 or 30,000, the probabilities are that one will find gangrene of the obstructed loops or beginning peritonitis. If observed on the second or third day after the beginning of the symptoms, it is difficult to make a differential diagnosis with regard to gangrene or peritonitis. After the third day in cases in which there is no gangrene and no peritonitis, or in which the auto-intoxication is not yet very grave, the leukocytes still remain high—15,000 to 23,000—according to the degree of obstruction—complete, higher; partial, lower. In the presence of gangrene, peritonitis, or grave auto-infection, the leukocytes begin to fall. If the patient is admitted after the third or fourth day, with a history of intestinal obstruction, and still has a high leukocyte count, the prognosis is good for operation. If the count is low, and especially if it is below 10,000 the probabilities are that on operation extensive gangrenous peritonitis will be found; or the patient will be so depressed by auto-intoxication that reaction does not follow relief of the obstruction.

The hyperleukocytosis is of the neutrophilic type and is associated with a decrease or absence of eosinophiles (septic factor).

The Stomach Contents.—Vomiting is a constant symptom of intestinal obstruction. At first there is nothing characteristic about the material that is brought up, but after a variable time a fecal odor becomes noticeable, which is referable to the entrance into the stomach of decomposing albuminous material from the intestinal tract. Formerly it was thought that fecal matter actually was vomited, but this, of course, is not the case in the sense that the contents of the lower bowel back up into the stomach. The term has reference to the fact that in intestinal obstruction, no matter where located, albuminous putrefaction develops already in the small intestine, giving rise to the formation of indol and skatol; when such material enters the stomach vomiting results, and with it the odor of fecal matter.

The Urine.—This is naturally much reduced in amount, high colored, and readily deposits urates on standing. In cases where the obstruction is located in the small intestine there is intense indicanuria, owing to the abundant amount of albuminous material in that section of the digestive tract which falls a prey to putrefactive organisms. The amount of phenol is similarly increased and the ratio between the conjugate and mineral sulphates correspondingly diminished. In one case of this order, in which obstructive symptoms had existed for ten days, I found the ratio 1 to 1.5. In obstruction of the large

intestine indicanuria of such intensity is not observed, possibly owing to the fact that there is not a sufficiently large amount of albuminous material available. In advanced cases albuminuria may be observed and with it the presence of casts, both hyaline and granular, in large numbers.

INTESTINAL PARASITIC DISEASES

(See Intestinal Helminthiasis)

KALA-AZAR

(Tropical splenomegaly; black fever)

Essential Factors.—Marked chlorotic anemia; leukopenia; large mononucleosis; increase of the plaques; presence of the Leishmania-Donovani in the splenic blood and in the pus from associated suppurative foci.

The Blood.—*The Red Cells and Hemoglobin.*—In all cases of Kala-azar a marked anemia of the chlorotic type develops sooner or later in the course of the malady. In part, no doubt, this is due to the disease itself, but it is probably intensified by the associated hookworm infection which is noted in almost all cases. Rogers' average count was 3,500,000. The oligochromemia, however, usually exceeds the oligocythemia. Morphological examination reveals a variable degree of poikilocytosis, polychromatophilia, and frequently the presence of isolated normoblasts.

The Leukocytes.—The leukocytes are usually diminished, the diminution affecting the neutrophilic elements, while the large mononuclears are increased. In a series of ten cases, reported by Donovan, the average was 21.58, with variations from 6 to 48 per cent. In one case mentioned by Neave, 67 per cent. were counted. Owing to the associated uncinariasis there is frequently also hypereosinophilia. In one case observed by Swan there were 5 per cent. of mast cells.

The Plaques.—The plaques are usually much increased.

The Leishmania-Donovani.—The diagnosis of the disease depends upon the demonstration of the corresponding parasite—the Leishmania-Donovani (which see). In the peripheral blood these bodies are rarely found and only when the temperature is high. Splenic puncture gives the best results. Donovan suggests that it is advisable to keep the patient flat on his back for twenty-four hours after the puncture and to give a dose of calcium chloride immediately after and twice again at intervals of three hours, in order to prevent hemorrhage. The parasites are principally met with in large mononuclear cells, but also occur free in the serum. The typical forms are oval or circular, with a well-marked contour (see illustration). There is a deeply staining nucleus lying against the capsule, and a deeply

staining rod-like centrosome. They may occur singly or in pairs, or in zoöglea masses. They are readily stained with any one of the methylene-azure mixtures (Hastings, Giemsa, Leishman). Their number is variable, and not infrequently quite considerable.

In some cases the disease is associated with malaria, when the corresponding parasite may likewise be demonstrated.

The Pus.—In the pus from the various suppurative lesions which may be observed in some of the cases (*tropical ulcer, Delhi boil, Aleppo button, Scinde sore, oriental sore*) the parasite may also be found at times. It is apparently also the causative organism of the naso-oral type of leishmaniasis, known as espundix, which occurs in South America (Peru and Brazil) and in the Anglo-Egyptian Sudan.

The Urine.—Regarding the urinary picture there are no available data.

LARYNGITIS

Aside from tubercular infections the organisms most commonly met with in cases of laryngitis and tracheitis are the Micrococcus catarrhalis and the Staphylococcus aureus (Hastings and Miles).

LEAD POISONING

Essential Factors.—Secondary chlorotic anemia; basophilic granular degeneration; variable hyperleukocytosis with normal differential count; general tendency to interstitial nephritis, with corresponding urinary changes.

The Blood.—*The Red Cells and Hemoglobin.*—Anemia of the chlorotic type develops in all cases of lead poisoning. Its degree depends essentially upon the intensity of the intoxication and its duration. The greater the chronicity, other things being equal, the more intense the anemia. Averages are accordingly of no special interest. In a series of cases which I have collected from the literature the red cells ranged between 1,300,000 and 5,130,000 and the hemoglobin between 25 and 79 per cent. It is interesting to note that with this comparatively moderate anemia nucleated red cells are quite frequently encountered; they are almost exclusively normoblasts, but occasionally an isolated megaloblast may be observed. In a measure characteristic is the almost invariable presence of stiple cells (basophilic granular degeneration), for, with the exception of pernicious anemia, there is no condition in which this is so constant. My own investigations, besides those of Grawitz, Bloch, White and Pepper, and others, prove that even a comparatively trifling exposure to lead almost invariably leads to the appearance of stiple cells in the blood. Generally speaking, their number is proportionate to the degree of intoxication, but it is noteworthy that in the gastro-intestinal

cases the number is greater and the size larger than in the neuropathic cases. In rare instances they may be absent. Occasionally some polychromatophilic red cells are also encountered. Very rarely Cabot's ring bodies may be seen.

The Leukocytes.—In some cases a hyperleukocytosis varying between 10,000 and 23,000 cells may be observed, while in others, even though active symptoms exist, the number is normal. As the differential count in those cases in which hyperleukocytosis is noted shows no essential increase of any one variety, the inference is warrantable that the general increase must be due to accidental factors, and is hence of no significance. In some of my own cases, without hyperleukocytosis, the lymphocytes were increased. Occasionally neutrophilic myelocytes are seen, the number varying between 0 and 4 per cent.

The Urine.—The demonstration of lead in the urine when this is present in small amount is a fairly laborious undertaking, and is only exceptionally attempted.

In chronic lead poisoning there is a marked tendency to interstitial changes in the kidneys, which lead to corresponding changes in the urine. (See Nephritis, Chronic Interstitial.) But even in acute poisoning or during acute exacerbations of chronic cases, even though no renal changes of moment exist, albuminuria is common; this is true especially of cases with lead colic. The amount is usually small, and after a decline of the acute symptoms the albuminuria disappears. A few hyaline and finely granular casts are common.

LEISHMANIASIS

(See Kala-azar.)

LEPROSY

Essential Factors.—Marked anemia in advanced cases, sometimes resembling the pernicious type; no increase of leukocytes; lymphocytosis; presence of the leprosy bacillus in the blood taken from the nodules.

The Blood.—*The Red Cells and Hemoglobin.*—Early in the disease and in mild cases throughout the greater part of its course there may be little, if any, anemia. In the later stages, however, and particularly in those cases in which there is extensive ulceration, the loss of red cells and hemoglobin is frequently very considerable. At times the blood picture closely simulates that of pernicious anemia, viz., there is an oligocythemia exceeding the oligochromemia, with increased color index and general tendency to macrocytosis; the number of the normoblasts, however, exceeds that of the megaloblasts. In others the anemia is of the chlorotic type.

The Leukocytes.—The leukocytes are not increased, and, as in uncomplicated cases of tuberculosis, there is a tendency to lymphocytosis and to a certain extent also to an increase of the large mononuclears. Boston has noted an eosinophilia of 8.7 per cent. in one case.

Bacteriology.—Several observers claim to have found the *leprosy bacillus in the blood* during life, while others report negative findings. *Agglutination* of the corresponding organism by the serum of the patient has also been described.

Presence of the Leprosy Bacillus in Tubercles.—In doubtful cases it is best to excise a suspected area, to transplant a portion of the tissue into a guinea-pig (in which in contradistinction to the result with tubercular material no infection will occur), and to examine sections of the remainder for the corresponding organisms. The presence of large numbers of bacilli lying in the interior of cells would be strongly suggestive of leprosy. Direct attempts at cultures with blood that has been aspirated from the suspected nodules should also be made. Bibb claims to have obtained positive results in all cases with this method, while general blood cultures have proved negative.

The Urine.—Regarding the urine there are no data.

LEUKANEMIA

The Blood.—In the chronic form of the disease there is a composite blood picture of secondary anemia and either small lymphocytic or myelocytic leukemia, while in the acute cases (Leube type) we find the blood picture of a pernicious anemia associated with that of leukemia. A recent analysis of the recorded cases shows that in a small number the pathological findings corresponded to the hematological picture and that the term leukanemia may accordingly be retained to indicate a definite entity. Other cases were evidently cases of primary and secondary bone-marrow tumors, or cases of Banti's disease (sc. Splenic Anemia).

The usual findings in the pure cases are shown in the accompanying table:

Author.	No. of red cells.	Hb. Per cent.	No. of leukocytes.	Small monos.	Large monos.	Polys.	Eos.	Mast cells.	Myeloc.
Leube-Arneth	250,000	10	10,600	4.9	35.3	44.1	...	few	13.6
Luce	1,652,000	35	81,000	8.2	36.0	53.0	2.3	...	0.5
Sacconaghi	1,340,000	32	11,000	19.7	9.6	55.0	2.7	1.3	4.4
Parkes-Weber	1,800,000	27	3,000	59.0	...	37.5	0.5	...	3.0
Morowitz	881,000	25 to 30	9,800	19.0	1.5	74.0	0.5	0.5	4.5

LEUKEMIA (ACUTE LYMPHOCYTIC)

Essential Factors.—Secondary anemia; hyperleukocytosis; macrolymphocytosis; increased elimination of phosphoric acid, uric acid, and xanthin bases in the urine.

The Blood.—The blood changes in acute lymphocytic leukemia are essentially the same as in the chronic variety. The *anemia* develops quite rapidly and is apt to become very severe. Nucleated red cells, in contradistinction to the chronic type, are often seen in large numbers; these are for the most part normoblasts, but in some cases megaloblasts also are common. The morphological changes affecting the red cells are otherwise the same.

The Leukocytosis.—The leukocytosis is variable in extent. While in most cases values equally high, as in chronic leukemia, occur at some period in the course of the disease, the examination frequently shows the existence of a moderate leukocytosis only, and in some cases the number does not exceed what we would expect in an ordinary inflammatory leukocytosis at any time. When septic complications supervene, leukopenia may develop. Fränkel thus cites a case in which the number fell from 220,000 to 1200.

The predominating cell in acute lymphocytic leukemia is usually, but not invariably, the large lymphocyte, the number of which frequently exceeds 80 per cent. But in some instances the small variety controls the blood picture, while in still others both types are represented in approximately equal proportion. Cases have been reported where the chronic type of the disease has changed to the acute form, and the small lymphocyte coincidently was replaced by the larger cell, and conversely it has been observed that an initially acute case subsequently pursued a more chronic course, while the large lymphocyte gave way to the small variety.

The other forms of leukocytes are greatly diminished in this type of the disease, as in the chronic form.

It is interesting to note that in the event of a complicating sepsis the lymphocytosis may not be replaced by a neutrophilic polynucleosis, as is commonly the case in the myelocytic type. This, no doubt, indicates that not enough myelocytic tissue remains in the bone marrow to bring about an appreciable response. Da Costa mentions a case of this kind, where with the development of sepsis the leukocytes fell from 40,000 to 5661 within a few days and further fell to 471 within nine days, the lymphocytes remaining above 90 per cent. In an acute case described by Wende a streptococcus infection occurred, and twenty-four hours before death the total count was 1600 with 88 per cent of lymphocytes.

Chemical Examination of the Blood.—This shows practically the same feature as in the other types of leukemia; the uric acid content, however, is apt to be still greater—as high as 22.6 mg. in 100 c.c. of blood.

The Urine.—The urinary changes are essentially the same as those observed in other forms of the disease. The acute course, however, commonly finds its expression in an increased elimination of nitrogen—up to 21 grams in the twenty-four hours—and the higher value of the

uric acid and xanthin bases. In a case which was studied by Magnus-Levy the daily output of uric acid rose to 12 grams and the xanthin bases to 0.321 gram. The phosphoric acid is then correspondingly increased. In the same case of Magnus-Levy, 15 grams of P_2O_5 were eliminated in fifteen hours. This, of course, is exceptional, but other observers have noted 5 to 7 grams on repeated occasions.

LEUKEMIA (CHRONIC LYMPHOCYTIC)

Essential Factors.—Secondary anemia; hyperleukocytosis; microlymphocytosis; increased elimination of phosphoric acid, uric acid, and xanthin bases in the urine.

The Blood.—*The Red Cells and Hemoglobin.*—These are markedly diminished in most cases, the oligochromemia usually exceeding the oligocythemia, so that a lowered color index is the rule. The tendency to anemia in this form of leukemia is, on the whole, more marked than in the myelocytic variety. Counts lower than 2,000,000 are not at all uncommon. In some instances the cells may drop to 1,000,000 or even lower. Morphological examination of the red cells shows essentially the same features which are seen in the myelocytic variety. Nucleated red cells, however, are usually far less abundant and may, indeed, be absent, even though there be a marked corpuscular anemia. Megaloblasts may be present, but as in the myelocytic disease the normoblasts are always in excess.

The Leukocytes.—The leukocytes are much increased in number, though not so extensively, on the average, as in the myelocytic type of the disease. Cabot's average was 350,000, as compared with 438,000 in the latter variety. Exceptionally they may increase to 1,000,000 or more (1,480,000 in one of Cabot's cases), while, on the other hand, many counts are under 100,000. The leukocytosis is referable exclusively to an increase of the small lymphocytes and in some instances to the simultaneous appearance of large lymphocytes. Generally speaking, the smaller cells predominate in the more chronic cases, and the large cells in those with an acute tendency. But in some cases of either variety both types may be represented in approximately equal proportion, besides transition stages between the two. The relative count usually shows values ranging between 80 and 90 per cent.; sometimes they are higher, rarely lower. The large mononuclear leukocytes and the various varieties of granulocytes are correspondingly diminished, both relatively and absolutely. A few myelocytes may be encountered, but they do not belong to the blood picture of the disease. Morphological examination of the leukocytes shows relatively little deviation from the normal form; the cells are usually well preserved, though occasionally a great many disintegrating cells may be found. Sometimes large numbers of the cells show one or more nucleoli, and in almost any field an occasional cell

may be seen in which the protoplasm is collected into little knobs on the periphery of the nucleus. Cells with two nuclei are occasionally seen, while mitoses are rare.

The Plaques.—The plaques are usually increased.

The General Character of the Blood.—The general character of the blood, so far as macroscopic appearance, coagulability, specific gravity, and alkalinity are concerned, is essentially the same as in the myelocytic type of the disease (which see). The same holds good so far as the content in uric acid and xanthin bases is concerned

The Urine.—The urine presents the same general features as those seen in myelocytic leukemia. In one case of the disease, reported by Askanazy, the Bence Jones albumin was encountered.

LEUKEMIA (MYELOCYTIC)

Essential Factors.—Secondary anemia; presence of notable numbers of normoblasts; general granulocytosis; myelocytosis; increased elimination of phosphoric acid, uric acid, and xanthin bases in the urine.

The Blood.—*The Red Cells and Hemoglobin.*—While anemia develops sooner or later in all cases of leukemia, it is not warrantable to classify the disease among the primary anemias, as has been done in the past. The disease unquestionably leads to anemia, but this anemia is secondary. In slowly progressing cases it is not rare to find the red count but little diminished, while a study of the leukocytes and the clinical examination show that the disease is already well established. In the majority of cases, however, the anemia is already quite well marked when the patient is seen for the first time. Generally speaking, the red cells increase during periods of improvement, while the leukocytes decrease, and *vice versa*. But there are exceptions to this rule, and it may happen that a decrease in the number of the leukocytes coincides in point of time with a marked loss of red cells, which may be so extensive that the blood picture resembles that of pernicious anemia, particularly as the color index may then be increased. Toward the fatal end the patients usually become quite cachectic. The average count in Cabot's series was 2,706,000, the highest 5,000,000, and the lowest 408,000. Similar figures have been obtained by other observers. Da Costa's average of 29 cases was 2,814,000; his highest count was 4,200,000, and the lowest 572,000.

The loss of hemoglobin is in a general way proportionate to the loss of red cells, or exceeds this somewhat, so that a normal or somewhat diminished color index results. Many of the recorded figures, however, are unquestionably incorrect, as accurate hemoglobin estimations with the usual methods are frequently impossible, owing to the marked turbidity which is caused by the presence of so many

leukocytes. Da Costa's average was 48.6, with variations from 24 to 70, giving a color index of about 0.86. An increased color index is rare, but may occur (*vide supra*).

Morphological examination shows that the red cells are of normal size, frequently with pale centres, and essentially of normal shape. Poikilocytes may be present in small numbers, but they do not properly belong to the blood picture of leukemia; the same holds good for macrocytes and microcytes. Stiple cells in small numbers are frequently present, but they are, in my experience, not numerous; other observers seem to be under the impression that they are common. Polychromasia of the non-nucleated red cells is usually not extensive, but very common in the nucleated ones. The presence of the latter constitutes one of the most constant factors of the blood picture of the disease. In well-developed cases they are probably always present, and usually so in large numbers. In 2 cases mentioned by Taylor the nucleated red cells varied between 60,000 and 70,000 per cb.mm. It is surprising to find so many of these cells in cases in which the anemia is not at all extreme. The greater number by far are normoblasts; megaloblasts, however, are not uncommon, and occasionally one meets with typical gigantoblasts. The protoplasm of most of these cells is distinctly polychromatophilic. The nuclei of the normoblasts, for the most part, show a stellate arrangement of the chromatin (Rädchenkerne), others are pyknotic; karyolytic, and karyorrhectic nuclei are uncommon. In almost every specimen, in which normoblasts are numerous, it is possible to demonstrate karyokinetic figures; these are certainly much more common in leukemia than in any other pathological condition (so far as the blood is concerned).

The Leukocytes.—The leukocytes are enormously increased during the active stage of the disease; figures are then commonly met with which are not equalled in any other pathological condition. At the time when the patient first seeks medical advice it is common to obtain a count of 200,000 or more, and as the disease progresses, much higher values may be met with; counts of 500,000 and 600,000 are not at all rare. Da Costa mentions a count of 1,046,000, and Cabot one of 1,072,000. Counts lower than 100,000 are the exception. While the disease is progressing the leukocyte count is practically constantly high or rising, while during intervals of improvement lower values may be observed. As a result of vigorous treatment with arsenic or with the *x*-rays, or even spontaneously, the number may drop to normal and even become subnormal. I have observed the case of a woman (treated with arsenic) in whom coincidently with marked general improvement the leukocyte count dropped below 4000 (reaching 2000) on several occasions, and remained about normal for periods varying between several weeks and several months; a number of the counts were below 3000. McCrae has

reported a similar case. Since the introduction of the *x*-rays in the treatment of the disease, by Senn, many similar observations have been made. If the patient were seen for the first time at such a period the diagnosis might readily be missed, unless a careful qualitative study of the leukocytes be made (see below). My own patient, just referred to, applied for and obtained life insurance during such an interval. It is not in all cases, however, that a drop in the leukocyte count is associated with general improvement, for in some there is no corresponding increase of the red cells, but, on the contrary, a further drop. A fall in the number of the leukocytes is further observed in intercurrent infections, such as typhoid fever, influenza, miliary tuberculosis, pneumonia, etc. Dock thus refers to a case in which during an attack of influenza the cells fell from 367,000 to 5000; in v. Limbeck's case of complicating pneumonia there was a drop from 140,000 to 43,500, and in Müller's septic case from 246,900 to 57,300. In nearly all the cases, however, no matter in what manner the drop has been caused, there is sooner or later a rise again, after which the disease pursues its course, possibly with further intervals of improvement of the blood picture, but more commonly in a more or less uniform manner, directly toward the fatal end. The rapidity with which the changes from bad to better and *vice versa* occur is often most surprising. In my patient, referred to above, there was a drop from 350,000 to 4000 in one month; in a case of Plehn's there was a fall from 149,000 to 3000 in about seven weeks, and in some of the *x*-ray cases and in connection with complicating infections the drop may occur even more abruptly, and be more extreme. Dock mentions one instance of this kind in which the cells decreased to 470.

A remarkable drop in the number of the leukocytes also occurs following the administration of benzol and in a number of cases a return to a practically normal blood picture has been described. The improvement, however, seems to be only temporary, and the treatment is not without danger.

While the hyperleukocytosis in leukemia is frequently so extensive that the diagnosis of the disease could be made from this factor alone, it should be remembered that it is really the qualitative change in the leukocytic formula and not the grade of the leukocytosis which is characteristic. Whereas in the ordinary septic hyperleukocytosis the increase is referable exclusively to an increase of the polynuclear neutrophiles, the hyperleukocytosis of leukemia is brought about by an increase of all the granular types, the number being further augmented by the appearance of the corresponding myelocytes which normally do not find their way into the circulation and which in other pathological conditions are only temporarily and exceptionally encountered in relatively small numbers. If the relative percentages of the different granular types are considered irrespective of the age

of the individual cell, it will be found that the neutrophilic and eosinophilic values are essentially normal, but that the absolute values are enormously increased; if, however, the relative values of the adult cells are compared with the corresponding normal values, it will be found that they are quite constantly diminished in the neutrophilic variety and very commonly so in the eosinophilic type. As regards the numerical behavior of the mono- as compared with the polymorphonuclear *mast cells* no data have been published. These cells, however, are absolutely increased in practically all cases, and in most instances relatively as well, the values commonly being between 5 and 10 per cent. and frequently higher. It is noteworthy that this increase of the mast cells may be demonstrable at a time when the disease is quiescent. In one instance the total number of the leukocytes had been 350,000; three months later I counted but 2080, of which, 10.9 per cent. were mast cells; still later they rose to 15 per cent. The same was noted during several subsequent remissions through which the patient passed. Lazarus mentions a case in which the percentage was 47. On the other hand, they have been reported as absent, but this unquestionably is very rare.

Ehrlich once thought that an increase of the *eosinophiles* was so constant in myelogenous leukemia that the diagnosis should be abandoned in its absence. In view of more recent advances in our knowledge regarding the pathology of leukemia this statement can have reference only to the myelocytic type of the disease, and here it unquestionably holds in almost all cases; there are exceptions, however, and I have elsewhere reported a case of this order in which they were practically absent. This case I am now inclined to view as a case of leukanemia. While the absolute values of the eosinophiles are almost always increased, the relative proportion of the adult cells is frequently, indeed usually, low normal, if not subnormal. It is usually quite normal and even maximal normal, however, if we include the eosinophilic myelocytes in the count; in some cases these outnumber the adult forms. Their presence in notable numbers is one of the most characteristic features of the disease.

Neutrophilic myelocytes are always present in large numbers while the disease is active. This is really the most characteristic feature of the disease and the one upon which the diagnosis is dependent. There is no other pathological condition in which a similar and equally lasting increase is observed. The number of neutrophilic myelocytes is often most remarkable and counts of from 50,000 to 100,000 per c.mm. are by no means exceptional. The average percentage in Cabot's series was 37.7, corresponding to a total of 162,000 leukocytes. In Cabot's series, at the time of the first counts, the average was 20.6 and the minimal and maximal values 7 and 44 per cent. respectively. While many of the neutrophilic myelocytes belong to the small (trachychromatic) variety, an equal or larger

number are of the large (amblychromatic) type; it is the presence of this latter variety, indeed, which may be regarded as characteristic of the disease. In addition there are often many mononuclear cells which contain no neutrophilic granules, but present a fairly abundant markedly basophilic protoplasm in which rather coarse orthochromatically basophilic granules can be distinguished, lying embedded in a vaguely granular basophilic matrix. These cells may be viewed as myelocytes of an earlier generation (myeloblasts), from which in turn the amblychromatic variety is derived (see classification of the leukocytes). Besides these cells, other fairly large mononuclear, non-granular leukocytes may be met with which are even more difficult to classify; they evidently do not belong to the common large mononuclear type of the normal blood, and are perhaps best viewed as a variety of the neutrophilic myelocytes which for some reason has been unable to form its specific granulation. These cells are not often seen, but in some cases they may appear after the patient has presented a perfectly characteristic myelocytic blood picture, and may then become the predominating cells of the blood. I have described a case belonging to this order, in which the patient at first showed a blood picture strongly suggestive of pernicious anemia, which then changed to one typical of myelocytic leukemia and finally lost nearly all the myelocytes, their place being taken by the large non-granular cells just mentioned, the total leukocyte count at the time being about 300,000. Ehrlich, Musser, and others have reported similar findings, which were usually observed late in the disease.

During periods of improvement the number of myelocytes, coincidently with the general drop in the number of the leukocytes, is much reduced; in some instances they may indeed disappear. Sooner or later, however, as the disease progresses they reappear and may become more numerous than before. When septic complications supervene the myelocytic blood picture may disappear entirely, being replaced by an ordinary hyperleukocytosis with the septic factor, but in some myelocytes may remain even then, though probably always reduced in numbers.

The small mononuclear leukocytes are sometimes absolutely increased, but always relatively much diminished in number (to below 5 per cent.). Large lymphocytes can usually be demonstrated and may sometimes even be fairly common; but they do not constitute an essential factor of the blood picture of myelocytic leukemia. Phlogocytes (Türck's stimulation, or irritation forms) also may be seen, but are likewise not essential. Typical large mononuclear leukocytes are very scarce or absent.

A study of the morphology of the various leukocytes shows many deviations from the normal. The most striking is the remarkable tendency to undersize which is seen in the neutrophilic and eosino-

philic polynuclear forms, and in some of the neutrophilic myelocytes. Many of these are no larger than the average lymphocyte. The mast cells likewise may be dwarfed, but, on the other hand, it is common to find cells of this order which are much larger than the average; abnormally large neutrophiles and eosinophiles are less common. The content in granules on the part of the different varieties may also be quite variable and, as I have already pointed out, it would seem that in some cases the cells lost the power of forming neutrophilic material altogether; mast cells may be found with so few granules that the simultaneously dwarfed forms resemble lymphocytes in their general appearance. Broken cells with degenerating nuclei and granules scattered about are also common, suggesting an increased mechanical vulnerability. Very striking is the appearance of neutrophilic myelocytes with double nucleus; this is not uncommon, while leukocytes in actual mitosis are in my experience rare.

The Plaques.—The plaques are usually markedly increased in myelocytic anemia.

General Characteristics.—On naked eye examination the drop of blood, as it flows from the puncture, looks opaque, and on standing, the leukocytes collect in tiny masses resembling bits of pus which float about in the fluid portion and which frequently looks abnormally dark. In extreme cases, when the red cells are much reduced in number, the drop may assume a milky appearance. German writers speak of an occasional resemblance to a mixture of chocolate and cream. Owing to the high degree of tenacity of the leukocytes it is difficult to make good spreads; large drops must be avoided. Coagulation time, according to some writers, is normal (Cabot), while according to others (Grawitz) it is increased; in extreme cases clotting may practically cease. The water content of the blood is increased, varying between 815.8 and 881 per cent.; the specific gravity is correspondingly low and may diminish to 1.036. During the active period of the disease the alkalinity of the blood is frequently diminished, while normal values may be found at other times.

Charcot-Leyden crystals may be observed in wet specimens which have been allowed to stand exposed to the air for twenty-four hours or longer. Formerly when such preparations were more commonly in use, the crystals were, no doubt, more frequently seen; they are of no diagnostic significance. In the circulating blood they are never found, but they have been encountered in the aspirated splenic fluid, in hemorrhagic pleural exudates, and in the ascitic fluid of leukemic patients.

Uric acid and xanthin bases occur in the blood in notable quantities, the former sometimes reaching 11 mg. pro 100 c.c. of blood. Albumoses have been repeatedly demonstrated, while true peptone in the sense of Kühne is absent. Nucleo-albumin has been demonstrated by Mathes.

The Urine.—The urine shows no special features which may be deemed characteristic, unless it be the greatly increased elimination of uric acid and xanthin bases which is referable to the large number of disintegrating leukocytes; quite frequently the uric acid separates out in crystalline form, giving rise to a voluminous sediment. Coincidently there is an increased output of phosphoric acid, referable, no doubt, to the same source. These findings, however, are not constant and depend to a great extent upon the activity of the disease. Of especial interest is the observation of Edsall that in those cases of chronic leukemia in which there is a response to *x*-ray treatment uric acid and purin bases are at once markedly increased. Nitrogenous metabolism, on the whole, is frequently unaffected while the patient is in relatively good condition, but sooner or later marked losses of nitrogen develop. Small amounts of albumin may be met with later in the disease; sugar is absent.

LIVER ABSCESS

Essential Factors.—Secondary anemia; hyperleukocytosis of the neutrophilic type; bacteriemia in the bacterial cases; presence of the corresponding pathogenic agent in the pus.

The Blood.—*The Red Cells and Hemoglobin.*—Liver abscess leads to anemia in all cases. The rapidity with which this occurs and its extent depend upon the underlying cause. The severer types are usually seen in connection with multiple abscess formation the result of a general septicemia. In amebic cases the co-existing colitis frequently obscures the actual anemia owing to concentration of the blood. In the Hopkins series of fifteen cases (reported by Futcher) the average red count was 4,250,000, and the corresponding hemoglobin value 66 per cent.; the lowest count was 2,600,000, and the highest, 5,600,000.

The Leukocytes.—While leukocytosis occurs in most cases, it is often not high, even though there may be a large amount of pus. When it does exist it may be difficult to determine to what extent the increased count is referable to the hepatic complications and to what extent to the underlying disease. In the multiple cases referable to generalized septicemia the count is often very high—up to 50,000 or more. In the Hopkins amebic cases, all of which were associated with colitis, the average count was 18,350, which exceeds the average count of uncomplicated dysentery cases by 7750 cells. In some of the latter, however, counts were met with which were materially higher than in the complicated cases (40,000 and 47,000), so that the degree of leukocytosis is not an essential factor in the diagnosis of the abscess cases. In two of the abscess cases the highest count was less than 10,000, while the highest figure in the series was 53,000. The low values are explained by the existence of a

dense wall of inflammatory tissue which prevents the absorption of necrotic material. The differential count in the cases of bacterial origin shows the septic factor, viz., a notable increase of the neutrophiles associated with a decrease or absence of eosinophiles. In the amebic cases, when active resorption is going on, the hyperleukocytosis is also of the neutrophilic type, but in contradistinction to the bacterial cases the eosinophiles are apt to persist. This point has not received proper recognition. In chronic cases without hyperleukocytosis the differential count also shows no increase of the neutrophiles. Ewing mentions an instance where the total count was 11,000 and where the differential gave 52 per cent. of mononuclears, and 48 per cent. of neutrophiles.

Bacteriology. In the bacterial cases bacteriological examination of the blood frequently reveals the presence of the corresponding microorganisms.

The Sputum.—In some cases of liver abscess the diagnosis is not made until perforation occurs into the lung. I well remember the first case of amebic liver abscess which was observed at the Johns Hopkins. Its existence had been strongly suspected and the liver had been repeatedly explored with needles, but the abscess was not definitely recognized until the peculiar appearance of the sputum (anchovy sauce) led to its examination and the demonstration of the ameba coli. (See Lung Abscess.)

The Abscess Pus.—When exploratory aspiration of the liver leads to the discovery of pus the pathogenic agent can usually be demonstrated. In the bacterial cases the organisms most commonly concerned are the colon bacillus, the staphylococcus aureus, and the streptococcus; in the amebic cases the ameba is found, while bacteria are probably always absent; and in hydatid cases echinococcus hooklets and pieces of membrane may be encountered.

The Urine.—The urine shows no changes which can be attributed to the abscess *per se*.

LUNG ABSCESS

Essential Factors.—Secondary anemia with neutrophilic hyperleukocytosis; purulent expectoration containing elastic tissue and fragments of lung parenchyma; presence of the ameba coli in abscesses perforating from the liver.

The Blood.—The blood picture in pulmonary abscess depends to a large extent upon the nature of the underlying condition. Almost always there is marked anemia and hyperleukocytosis of the neutrophilic type. As the condition is so constantly a phase of a generalized septic infection, it is difficult to say to what extent the leukocytic picture is referable to the pulmonary complication *per se*, and it is hence of little interest from a diagnostic standpoint. The numerical values vary widely—between maximal normal values, on the one hand and 60,000 on the other.

The Sputum.—The diagnosis of abscess of the lung rests partly upon the clinical history and physical signs, but to a large extent also upon the character of the sputum and especially upon the demonstration of pieces of lung tissue or elastic fibers. The sputum as a whole is purulent, and on standing shows no marked tendency to separate out in layers; this occurs only after a time, when an upper frothy, watery layer appears above the purely purulent portion. The odor at first is insipid, but subsequently, when the abscess cavity becomes infected from without, it is apt to become fetid. Microscopic examination shows the presence of enormous numbers of pus corpuscles in all stages of degeneration, free fat globules, hematoidin partly in amorphous form and partly crystalline, plates of cholesterin (especially in cases of long standing), fatty acid needles, more rarely tyrosin and leucin. At first the primarily offending bacteria will be found without extensive admixture of others, but after a while the flora becomes quite diverse and luxuriant. The elastic fibers not infrequently present an alveolar arrangement, and in larger pieces of lung tissue the remains of bloodvessels can be distinguished.

The findings as above outlined have reference more particularly to large single abscesses. The small multiple type frequently goes unrecognized, as there may be little or no sputum to attract attention.

When a *liver abscess* perforates into the lung the sputum usually is quite characteristic. Its appearance (brownish red) is generally likened to that of anchovy sauce, or it is colored yellow by the bile pigment. Frequently the diagnosis is thus made by simple inspection. The amount of material which is brought up in these cases is often very large; it may indeed exceed a liter. At times it is brought up in paroxysms, the cavity being emptied of the greater part of its contents at one time. Microscopic examination shows the same constituents which are found in primary pulmonary abscess; in the hydatid cases, furthermore, pieces of membrane and hooklets, and in amebic cases the corresponding organism. In recent cases these may be fairly numerous, but in the more chronic forms the search is often tedious. I remember an instance of this kind, which I observed at the Hopkins Hospital as a young assistant, when the naked eye appearance of the sputum led me to an hour's but successful search. Sometimes, though rarely, bits of liver tissue are found in which the liver cells can be recognized as such.

In cases of *perforating empyema* the diagnosis is essentially made upon the basis of the physical examination, coupled with the sudden expectoration of a large amount of pus, containing relatively little elastic tissue and usually no lung tissue as such. The odor at first may resemble that of old cheese, but after a while, when the cavity has become infected from without, it becomes fetid. Otherwise the sputum presents no special characteristics.

LUNG (EDEMA OF)

The Blood.—The blood picture presents no characteristics which could be attributed to the edema *per se*, unless it be an occasional relative polycythemia.

The Sputum.—The sputum is essentially composed of blood serum tinged to a greater or less extent with hemoglobin. It is frothy and abundant, so long as the patients are still able to expectorate. When this point has been passed it often dribbles passively from nose and mouth, as Emerson very appropriately remarks, "one of the most gruesome sights of the sickroom."

MALARIA

Essential Factors.—Tendency to hypoleukocytosis; absence of the septic factor; tendency to splenocytosis; presence of the malarial organism in the blood; occurrence of pigment in the leukocytes.

The Blood.—*The Red Cells and Hemoglobin.*—Considering the fact that every red cell which has been invaded by a malarial organism will undergo destruction at the expiration of the period of time which is necessary for its full development, it would seem to follow that the degree of anemia which is produced will be directly proportionate to the number of the infected cells. While this is true in a general way, we find that the loss of corpuscles actually exceeds the number of infected cells, so that we are forced to the conclusion that a certain degree of hemolysis must be produced indirectly. The extent to which this second factor is operative is variable, and sometimes very material.

The greatest loss of red cells is brought about during the first paroxysms, and it is astonishing to see the extensive destruction which even a single attack can then produce. Kelsch states that the loss on the first day of the disease may amount to 1,000,000 cells pro c.mm. and that 2,000,000 cells may be destroyed within the first four days. Later on, owing to the development of a certain degree of immunity, no doubt, the destruction is less extensive. The greatest loss apparently occurs in estivo-autumnal cases, where the number of red cells generally falls to 2,000,000 or even lower. In chronic cases a definite cachexia develops and the count may fall to less than a million. Very curiously marked anemia may also develop in non-febrile cases of so-called larval malaria, and Ewing states that he found a further progress of postfebrile anemia to be the rule in all severe cases, and that in some instances it proved fatal.

Early in the disease, and especially in the tertian and quartan forms, blood regeneration is exceedingly active; so much so, in fact, that the loss incurred during one paroxysm may be fully made up

by the time that the next one develops. Later on, however, the bone marrow becomes less responsive, and in chronic cases, of estivo-autumnal infection more particularly, the resultant anemia may be very persistent.

The *hemoglobin* is generally reduced *pari passu* with the red cells, so that the color index is practically normal. Exceptions, however, are common. On the one hand the loss of hemoglobin may be greater than that of the red cells, while on the other the blood changes, with increased color index, may closely simulate those of pernicious anemia. During convalescence the regeneration of the red cells commonly proceeds more rapidly than the reformation of the hemoglobin, so that a low color index is the rule during this period.

Nucleated red cells, of the normoblastic type, are frequently seen when marked anemia exists, and they may be quite numerous. Megaloblasts are rare; they are essentially found in pernicious cases with profound anemia.

Morphological Changes in the Red Cells.—Examination of the wet specimen shows evidence of the existing anemia by the pallor of the corpuscles, and in well-developed cases poikilocytosis and a certain degree of anisocytosis will be noted.

A curious difference exists in the effect of the species of the parasite upon the size and color of the red cells. In tertian fever they become progressively paler and larger, while in the estivo-autumnal and quartan types the cells are apt to diminish in size, at the same time assuming a peculiar brassy appearance, which is very characteristic.

In stained preparations a variable degree of polychromatophilia may be observed. The bronzed cells stain especially deeply in the color of the common red cells and not necessarily polychromatically. It would seem as though the condition were due to a concentration of the hemoglobin (an erythropyknosis, as suggested by the Italians). It is usually stated in text books that basophilic granular degeneration of the red cells (type Grawitz) is common in malaria. My own experience, which is based upon the study of a large amount of material, leads me to believe that this is an error. Stipple cells do occur, but they are not usually numerous. It is possible, as Grawitz has suggested, that the basophilic granules which Plehn reported as being so common in European newcomers in the tropics, and which he viewed as an early phase in the development of the malarial parasite, and which Grawitz interprets as the ordinary type of granular degeneration, are referable to the action of the tropical climate *per se*. They certainly have nothing to do with the malarial organism, and it is unwarrantable to bring the two conditions into association. A different type of stippling, however, is frequently seen in cells which are infected with the tertian parasite. This has been described especially by Schüffner, and the granules are generally spoken of as Schüffner's granules. They stain red with polychrome dyes

containing methylene azure, and are frequently so numerous as almost to hide the parasite from view (Plate XII, Fig. *c*).

The Leukocytes.—While the leukocytes are slightly increased in number immediately preceding and during the first hours following the chill, there is subsequently a distinct tendency to hypoleukocytosis. In Billings' series the average count was 4323, and in that of Da Costa, 5622, the lowest values being usually found at the end of the paroxysm when the temperature is subnormal (average 2300). A material difference does not seem to exist in this respect between tertian and estivo-autumnal fever. The average of 82 cases of the estivo-autumnal type observed at the Johns Hopkins Hospital was 3500, and of 70 tertian cases, 4500. The highest values are seen in pernicious cases. Emerson mentions an instance in which a count of 50,000 was obtained one hour before death.

The differential count shows absence of the septic factor and a distinct increase of the large mononuclear elements, which is most pronounced during the apyrexial period. The values are usually above 15 and may attain 40 and 50 per cent. This increase is frequently of diagnostic importance, particularly in cases in which, as a result of the administration of quinine, the parasite may be temporarily absent from the peripheral circulation. I would emphasize however, that it is not advisable to attach too much importance to this symptom, as a large mononucleosis is by no means infrequent in diseases which are not malarial. In suspected cases it is well to watch for the presence of brown pigment granules in the large mononuclear elements. Their demonstration may be regarded as equivalent to the demonstration of the parasite, from which they are in fact derived. The polynuclear leukocytes are less important in this respect, as the large mononuclears are the true scavengers in malaria. When present, the pigment usually occurs in little blocks, which often lie in small vacuoles; the same cell may contain a number of such blocks (Plate XII, Fig. *f*).

The small mononuclear leukocytes play no essential role in malaria, and Krauss very properly emphasizes that it is not so much the absolute increase of the large mononuclears which is so constant in malarial infection, as the relative increase over the small mononuclears.

The neutrophiles are proportionately diminished, excepting during the pyrexial stage, when they usually assume normal or maximal normal values. Metamyelocytes in small numbers are occasionally seen, but are more common in cases associated with marked anemia.

From the few available data it is not possible to draw any conclusions regarding the karyomorphism of the neutrophiles. Normal findings as well as a tendency to monokaryolobism have been reported.

It is generally stated that the eosinophiles are present in increased numbers during the afebrile period of the disease, and that they rarely

diminish below minimal normal values, even at the time of the paroxysm. Zappert reports a case in which on the day following the last attack 20.34 per cent. (1486 absolute) were found. Krauss, on the other hand, in an analysis of 204 cases, in nearly all of which the organism could be demonstrated, found but 2 per cent. on an average, and supernormal values only exceptionally. Fontaine, who has had a very extensive experience in the study of this question in Louisiana, tells me that in uncomplicated cases of malaria eosinophilia is rare. This I have been abundantly able to confirm from a survey of the large number of specimens which I owe to his courtesy.

Occurrence of the Malarial Parasite in the Blood.—If proper search is made the specific parasite can be demonstrated in the blood in all cases of active malaria. The readiness with which it is found, however, is dependent upon several factors, notably the type of the infection, the time of the examination in reference to the last chill, and the administration of quinine. Generally speaking, the best results are obtained about midway in point of time between the occurrence of two consecutive chills. Immediately after an attack the findings will frequently be negative, as the youngest forms only will be present (unless a double infection has occurred), and many of these may not as yet have entered red corpuscles. Full-grown forms usually occur only at the beginning of or during a paroxysm, and it might hence be argued that this would be the best time for a search. In tertian fever or the quotidian form, depending upon double infection with the tertian parasites, the most beautiful pictures are indeed obtained at this time, and in the wet preparation it is possible to observe the entire process of segmentation and frequently of exflagellation. In the estivo-autumnal type of the disease, however, the peripheral blood may contain no parasites whatever early in the attack, owing to the fact that sporulation in this form occurs in the internal organs. In such cases it is advisable to wait for some ten or twenty hours after the paroxysm before taking the blood.

While the above are general rules, it should be remembered that definite clinical symptoms will only occur, if a sufficiently large number of organisms are present and segmenting at the same time (250,000,000 according to Ross), and that segmentation in the estivo-autumnal form may occur at irregular intervals after a time, so thatan originally intermittent fever may thus be transformed into a more or less continued fever.

In patients who have taken quinine the search for the parasite is usually more or less tedious. This is true especially in cases of tertian infection, while the parasites of quartan and estivo-autumnal fever are more resistant.

Regarding the question whether fatal cases of malaria can occur without parasites in the peripheral blood, the evidence goes to show that this is very unlikely. Coma, arising in the course of the active

sporulating cycle, is, according to Ewing, almost invariably fatal, and he regards the presence of many ameboid parasites in the blood of such cases as an extremely unfavorable sign. He finds that in most cases of malarial coma, especially in those of very abrupt onset, or with symptoms of meningitis or localized cortical irritation, crescents only are found in the peripheral blood, and in these recovery usually follows.

The number of parasites which may be found in the peripheral blood at one time is, of course, quite variable. In quiescent cases it may be necessary to examine many slides before the first organism is found. In active cases which have had no quinine they are usually quickly found, and while in some only one organism will be demonstrated in several fields, many will show one or more in a single field, and in others, particularly in severe infections with the estivo-autumnal form, from 10 to 30 or more are present; in the latter event it is not uncommon to find 2, 3, or more in a single corpuscle. Sexual forms are rarely encountered until the disease has existed for some time, viz., until several generations have developed asexually.

The Urine.—The condition of the urine in malarial fever will depend to a great extent upon the existence or non-existence of fever, the intensity of the infection, the extent of blood destruction, etc. As a general rule, during the febrile period the color is higher than usual and the specific gravity increased. The amount may be normal, slightly diminished or slightly increased (700 to 1300 c.c.); with convalescence a rise is often observed, when previously it had been diminished. The reaction is markedly acid during the febrile period. Sediments of urates are common.

Regarding the elimination of urea the results obtained by different observers are not in accord, for while according to some the amount is diminished, others report an increase during pyrexia, which may amount to 45 grams in the twenty-four hours. The uric acid tends to increase on the days of the paroxysms. The chlorides may be diminished or increased, but are usually normal. The phosphates are normal or slightly diminished. The indican is frequently increased to a very marked degree. Uroerythrin is constant and quite abundant. Urohematin and hydrobilirubin (urobilin) are both increased. Sugar is absent. A moderate amount of albumin is present in many cases; at the Hopkins in 46.4 per cent. Other observers have reported albuminuria still more frequently (up to 75 per cent. of the cases). Acute nephritis develops in about 5 per cent.

Malarial hematuria and hemoglobinuria are common in the tropics of Africa (blackwater fever) and in certain districts of the Southern States, while they are rare in the more temperate zones. The condition usually occurs in individuals who present a well-marked cachexia. The parasite cannot always be demonstrated in the blood at the time, and it is noteworthy that in certain districts the

hemoglobinuria develops after the true malarial season is over. Urobilinuria is more frequent than hemoglobinuria and, as would be expected, especially intense in the latter condition.

The diazo reaction is uncommon. The amount of acetone is normal in the interval, but somewhat increased during the febrile period; not so much, however, as in the continuous fever.

MALTA FEVER

Essential Factors.—Chlorotic anemia; no increase of the leukocytes; large mononucleosis; presence of specific agglutinins and of the corresponding organism in the blood.

The Blood.—*The Red Cells and Hemoglobin.*—Anemia of the chlorotic type is observed in practically all cases, and is, generally speaking, proportionate to the intensity of the infection. It is most pronounced at the termination of the febrile period. The lowest values are found in the hemorrhagic cases. Basset-Smith speaks of an average erythrocyte loss, ranging between 20 and 40 per cent. below the normal standard, with a relatively greater loss of hemoglobin.

The Leukocytes.—The leukocytes are not increased, unless complicating factors, such as hemorrhage, supervene, in which case a moderate increase may be observed temporarily. Some writers report that there may be a leukopenia, as in typhoid fever. The differential count shows a marked increase of the large mononuclears (26 to 76 per cent.).

Agglutination Reaction.—The diagnosis of the disease has been greatly facilitated by the discovery that the serum of the patients causes clumping of the corresponding organism—the Micrococcus melitensis. As a rule, a dilution of 1 to 50 and a time limit of one-half hour should be employed. (See Technique.)

Bacteriology.—According to Gilmour, Shaw, Basset-Smith, and others, the Micrococcus melitensis may be demonstrated in the blood by culture, in practically all cases. It is most likely to be found during the early stages and in severe febrile relapses, while in the afebrile intervals and the subsequent cachexial stage it is not found. In no case, however, are the organisms abundant, and for this reason the bacteriological findings are at times rather uncertain.

The Urine.—Regarding the condition of the urine there are no adequate data.

MANIC-DEPRESSIVE INSANITY.

The Blood.—*Serology.*—In manic-depressive insanity, in contradistinction to dementia præcox, the Abderhalden reaction with genital tissue is negative.

MEASLES

Essential Factors.—Hypoleukocytosis, lymphocytosis, diazo reaction.

The Blood.—*The Red Corpuscles and Hemoglobin.*—In uncomplicated cases the destruction of red cells amounts to not more than 250,000 to 500,000 corpuscles, with a loss of from 15 to 20 per cent. of hemoglobin. Complicating conditions, of course, may lead to varying degrees of anemia. Morphological changes are usually absent.

The Leukocytes.—Measles, like typhoid fever, is a notable exception to the general rule that the acute infections are associated with hyperleukocytosis. There is, on the contrary, a very distinct tendency toward leukopenia. This, however, is preceded by a preeruptive increase which commences at the beginning of the period of invasion, then increases rapidly and reaches its maximum about the sixth day before the appearance of the rash. After this the number diminishes, and at the appearance of the eruption and during its course the occurrence of an increased number of leukocytes indicates a complication. The leukopenia generally reaches its lowest point on the second day of the rash, when the number is usually reduced to about one-half of the normal. After this the cells increase again, more or less rapidly, and reach the normal line one to five days after the disappearance of the rash. The early hyperleukocytosis is due to a moderate increase of the neutrophiles; these then diminish to minimal normal or subnormal values, while the lymphocytes are, relatively at least, increased; this increase is notably seen in cases with marked adenitis and diarrhea. The eosinophiles are either absent or much diminished during the active stage of the disease. With the appearance of convalescence they return to normal, while the neutrophiles also gain their former level and may even be increased.

The Arneth count at the height of the disease shows a marked diminution of the polynuclear forms; there is thus, as in typhoid fever, a marked anisohypocytosis.

The Plaques.—The blood platelets are much diminished at the height of the disease (62,000), while they rise again after the disappearance of the fever.

Bacteriology.—The bacteriological examination of the blood shows nothing in uncomplicated cases. Jehle as well as Canon claim to have found influenza bacilli during an influenza epidemic.

The Urine.—During the active period of the disease the urine presents the usual febrile characteristics. The *diazo reaction* is quite common. Rivier found it in 75 of 85 cases.

MENINGITIS (CEREBROSPINAL, EPIDEMIC TYPE)

Essential Factors.—Hyperleukocytosis; septic factor; presence of the meningococcus in the blood and in the cerebrospinal fluid; neutrophilic polynucleosis of the cerebrospinal fluid.

The Blood.—*The Red Cells and Hemoglobin.*—Considering the lightning course which epidemic cerebrospinal meningitis takes so frequently, it is not surprising that the red count and hemoglobin values show no material change in many of the cases. When the disease pursues a slower course a certain grade of anemia will sooner or later develop; this, however, is usually mild and does not attract special attention.

The Leukocytes.—These are materially increased in practically all cases. There are few bacterial infections, indeed, in which higher counts or counts of equal height are observed. Koplik thus found values exceeding 35,000 in 55 per cent. of his cases, the general range being between 12,000 and 55,000. Initial values lower than 10,000 are exceptional; in the series of 181 cases collected by Cabot such figures were only noted in 9. Unfortunately no statement is made regarding their severity and the time of observation; probably they were counts taken later in the disease. Generally speaking, the height of the leukocytosis is proportionate to the intensity of the infection, but it is to be noted that a high count does not necessarily imply a fatal ending. The differential count shows the septic factor, viz., a large increase of the neutrophiles with absence or diminished number of the eosinophiles. Occasionally this is relatively obscured by the appearance of numbers of large mononuclear non-granular cells, which occurring in the blood are not unnaturally counted as large mononuclear leukocytes. My own impression has been that these cells are endothelial cells and not leukocytes. I have observed them in two cases of the disease, and in both I could also demonstrate the presence of the meningococcus in the blood by direct microscopic examination (see below). During the febrile recrudescences, so common in chronic cases, the leukocytes usually rise again, though the eosinophiles may not disappear, as they ordinarily do in the earliest days of the illness (Cabot).

Presence of the Meningococcus in the Blood.—The meningococcus has been repeatedly found in the circulating blood by cultural methods, and in several cases it has been demonstrated directly by the microscope. I have found the organism twice in this manner, both cases being very severe and ending fatally. In one of the cases I calculated that 7,380,000 meningococci were present in 1 c.c. of blood. Almost all were inclosed in polynuclear neutrophiles and in the large mononuclear cells, referred to above, which I was inclined to view as endothelial cells. In a recent fatal case, occurring at the Mercy Hospital, Skilton likewise found the organism

directly in a smear. In several other cases which I had a chance to observe I did not find the organism.

The Cerebrospinal Fluid.—The cerebrospinal fluid in meningococcus meningitis varies somewhat in appearance. Not infrequently it is quite clear, or but slightly cloudy, but in other cases it is markedly turbid, and in some of the fatal cases a thick, pus-like fluid is encountered. The amount varies correspondingly. As a rule, 70 to 80 c.c can be obtained quite readily, while in others it is less, and in exceptional cases none is found. The specific gravity is high (1.010 to 1.012).

Cytological Examination.—This usually shows the presence of large numbers of leukocytes, the polynuclear neutrophile being the predominating cell, excepting in chronic cases where lymphocytes prevail. These cells also enter into the foreground as recovery occurs.

The *meningococcus* can be demonstrated in a large number of cases. Councilman states that during the Boston epidemic, a few years ago, lumbar puncture was performed in 55 cases, and that the organism was found on microscopic examination or by culture in 38. It was present in all the acute cases, but rarely found in those pursuing a more chronic course. The average time from the onset of the disease before spinal puncture was made was seven days in the positive and seventeen in the negative cases. The longest time after the onset at which a positive result was obtained was twenty-nine days. Similar results have been reached by other observers. Koplik found the organism within the first twenty-four hours after the onset of the disease and as late as the fifteenth week. But, like Councilman, he also found that in the chronic cases, especially in those of the posterior basic type, it may escape detection. While at times the organisms can only be demonstrated by culture, they can usually be found in ordinary smears, if careful search is made. Frequently they are numerous. Some are found free in the fluid, but the majority are usually inclosed in polynuclear neutrophilic leukocytes. Their number may here vary considerably; on the one hand only one or two may be present in a cell, while in others they may be so closely packed as to obscure the nucleus. At times they may be present in enormous numbers; in one instance I found extracellular groups composed of hundreds of organisms.

Mixed infections are not uncommon in epidemic cerebrospinal meningitis. Councilman found the pneumococcus in 7 cases and Friedländer's bacillus in 1. Terminal infections with staphylococci and streptococci also occur.

Effect of Treatment with Antimeningococcus Serum.—In those cases which are favorably influenced by the antiserum there is a fall, often very rapid and even critical in the number of the leukocytes in the circulating blood, which goes hand in hand with the disappearance of the diplococci, and clearing of the spinal exudate.

The organisms (unless they were already absent) tend to become wholly intracellular, to present certain changes in appearance, as swelling and fragmentation, and to stain diffusely and indistinctly, and coincidently to lose their viability in culture (Flexner and Jobling).

The Urine.—In some of the cases there is very curiously marked polyuria even at a time when the temperature is high. This, indeed, is more common than oliguria, which is observed in others. Albuminuria with cylindruria may occur in the severest cases. Glucosuria has also been noted in some.

MENINGITIS (TUBERCULAR)

Essential Factors.—Irregular hyperleukocytosis; general tendency to normocytosis; cerebrospinal lymphocytosis; presence of the tubercle bacillus in the meningeal fluid.

The Blood.—*The Red Cells and Hemoglobin.*—All cases of tubercular meningitis are associated with a gradually developing anemia which is usually of the chlorotic type. Owing to a concentration of the blood as a whole, the actual findings, however, do not represent the true grade of anemia (see pulmonary tuberculosis).

The Leukocytes.—It is generally stated that in tubercular meningitis there is, as a rule, no increase in the number of the leukocytes. Early in the disease this is probably true, but later it is not at all uncommon to meet with higher values. To what extent this hyperleukocytosis is due to associated infections is difficult to say, but its occurrence lessens the value of the count in the differential diagnosis of the disease. Nevertheless, it may be stated, as a general rule, that a normal number, other things being equal, points to a tubercular in contradistinction to a purulent meningitis. In Cabot's series of 43 cases the counts varied between 2000 and 52,000, and it was higher than 10,000 in 32. In this series were 25 children in whom the tendency to hyperleukocytosis is always more marked than in adults. In the remaining cases, and throwing out those in whom death is reported to have occurred on the day of the initial count, the figures varied between 2000 and 15,300, and in these (12) the count was higher than 10,000 in only 5. Commenting on the occurrence of hyperleukocytosis in tubercular meningitis, Ewing remarks that he has seen such cases, but that a complicating terminal pneumonia was found at the autopsy in every instance.

Unfortunately differential counts are not available in any large series, so that it is impossible to lay down any definite rules. Hoagland reports 4 cases (children), in all of which the polynuclear neutrophiles were increased (82 to 90 per cent.) and the eosinophiles low (0.3 to 1 per cent.).

The *fibrin* is not increased.

The Cerebrospinal Fluid.—In the diagnosis of tubercular meningitis the examination of the cerebrospinal fluid is much more important than that of the blood.

The amount will depend upon the degree of intracranial pressure, but is naturally large (60 to 80 c.c.). The fluid is clear and, as a rule, colorless, unless a small bloodvessel has been punctured, when it may present a slight reddish tint; sometimes it is straw colored. On standing, very delicate coagula develop, which, like spider webs, extend throughout the fluid. The specific gravity may vary from 1.005 to 1.010. The reaction is alkaline. The amount of albumin is large, varying from 1 to 3 pro mille.

The cytological formula shows a marked preponderance of lymphocytes over polynuclear neutrophiles. This constitutes one of the most important factors in the differential diagnosis of tubercular from purulent meningitis, excepting in chronic cases of the latter type, where lymphocytosis replaces the original polynucleosis.

The tubercle bacillus can be demonstrated in many cases, if suitable methods are employed (which see). Fürbringer thus found the organism in 30 cases out of 37. Schwarz states that he obtained positive results in 16 cases out of 22. Slowyk and Manicatide found bacilli in all of 19 cases (sixteen times by direct microscopic examination, and three times by animal experiment), and Koplik found them in 13 out of 14 cases, using centrifugalized material.

The Urine.—The urine shows no changes which are characteristic.

MONILIASIS

(See Sprue.)

MUMPS

Essential Factors.—Lymphocytosis of the blood, and in cases complicated by meningitis, also of the cerebrospinal fluid.

The Blood.—According to Feiling there is a lymphocytosis in mumps which is both relative and absolute, leading to a slight increase in the total number of the leukocytes. The lymphocytosis is present already on the first day and persists for at least a fortnight. The occurrence of orchitis does not alter the leukocytic picture. In the small number of cases which I have had occasion to examine myself, the total number of leukocytes was not increased, but there was evident a distinct tendency to lymphocytosis.

The Urine.—The urine usually shows no special abnormality, but albuminuria is at times observed.

The Cerebrospinal Fluid.—In cases which are complicated by meningitis or by lesions affecting the cranial nerves there is a lymphocytosis also of the cerebrospinal fluid.

MYELOMATOSIS

Essential Factors.—Irregular secondary anemia; irregular leukocytic findings; Bence-Jones albuminuria.

The Blood.—*The Red Cells and Hemoglobin.*—Adequate blood examinations have been made in only a few of the recorded cases. From the available data it appears that more or less severe anemia is fairly common, but not at all constant. In several instances the red count has been found well below 3,000,000 (1,588,000 to 2,750,000), with a corresponding drop in the hemoglobin (15 to 50 per cent.). Exceptionally the picture resembles that of pernicious anemia. In one instance recorded by Gluzinski and Reichenstein the red count fell to 670,000. Nucleated red cells of the normoblastic type may be present in small numbers, and the last mentioned writers speak of the occurrence of megaloblasts.

The Leukocytes.—The total number of the leukocytes is usually normal or moderately increased (4500 to 15,000); higher values are exceptional (39,400). Differential counts have been recorded in only a few cases and show no uniformity. Voit-Salvendi found 60 per cent. of lymphocytes. Gluzinski and Reichenstein speak of the presence of 72 to 91 per cent. of mononuclear elements, which they interpret for the most part as plasma cells. Other writers mention the occurrence of a small number of myelocytes, while exceptionally a great many may be encountered (21.8 per cent., Sternberg). Still others state that they have met with no material deviation from the normal.

The Urine.—In many cases of myelomatosis, notably when affecting the thoracic skeleton, the so-called Bence-Jones protein is encountered in the urine. Its presence is virtually pathognomonic of the disease, as it is very rarely met with in other pathological conditions. A few exceptions, however, have been noted. Fitz mentions its occurrence in a case of myxedema, Askanazy, Ellinger, and Decostello in isolated cases of lymphatic leukemia, while still others have found it in rare instances of bone-marrow tumors of other kinds, viz., in endothelioma, chondrosarcoma, in extensive carcinomatosis affecting the bone-marrow, secondary to cancer of the stomach and of the breast, as also in one case of gunshot wound of the leg.

The amount of the substance which may be found in the urine is variable. Some observers have noted an elimination of from 0.25 to 6 pro mille, while others report much larger quantities. In Bence-Jones' case the elimination rose on one occasion to 6.7 per cent., corresponding to a total output of 70 grams in the twenty-four hours, *i. e.*, to nearly as much as the entire amount of the albumins of the blood plasma.

It has been reported by several observers that the Bence-Jones albuminuria was accompanied by ordinary albuminuria. In no case,

however, was the presence of common albumin established in a satisfactory manner, and it appears to me that its presence was merely assumed, whenever the urine did not clear entirely on boiling (see tests). This is unwarrantable, as it is now well known that the Bence-Jones protein itself, after being precipitated by heat, may not dissolve altogether on boiling. In two such cases, where one might have been led to assume the existence of ordinary albumin, I could demonstrate conclusively that this was not present. I should recommend that in all such cases the urine be carefully and slowly heated to 56° C., and maintained at that temperature until no more albumin separates out, and that on cooling it be filtered. The filtrate can then be tested as usual for common albumin, either by heat or other tests, and I think that it will be found that common albumin is not present. That the two conditions *may* occur together is, of course, *a priori*, possible, but in the previously recorded cases no satisfactory evidence has been brought forward to show that it did occur.

(For a description of the albumin in question see Part I.)

In other respects the urine shows no essential abnormality.

MYXEDEMA

Essential Factors.—Anemia; irregular leukocytosis with lymphocytosis and hypereosinophilia; presence of protective ferments in the blood; tendency to albuminuria.

The Blood.—*The Red Cells and Hemoglobin.*—In the majority of cases of myxedema there is a certain degree of anemia; usually this is moderate, but in some instances it is severe. Le Breton thus records an instance where the red cells numbered only 1,750,000. In most cases the anemia is of the chlorotic type, but in some the loss of red cells exceeds that of the hemoglobin, so that an increased color index is obtained. In Le Breton's case this was 1.91. Correspondingly, Kraepelin mentions that he found a distinct macrocytosis (8 to 14 μ) in several cases, associated with the presence of nucleated red cells (normoblasts). Similar observations are reported by Vaquez, while Cabot did not find an increase in the size of the red cells.

In one case Emerson found the plaques much increased.

The Leukocytes.—The leukocytes are not increased, as a rule, but in some cases a moderate hyperleukocytosis has been observed. There is usually a marked lymphocytosis (36 to 48 per cent.) and moderate hypereosinophilia (5 to 10 per cent.). A few myelocytes may be seen in anemic cases.

The Specific Gravity.—The specific gravity of the blood is increased (1.062 to 1.063 for the whole blood and 1.031 to 1.032 for the serum); the solids are correspondingly high.

Effect of Thyroid Treatment.—As a result of thyroid treatment in appropriate dosage the anemic cases show a material increase in the number of the red cells and leukocytes, while the hemoglobin is but little affected. In the case of Le Breton (above mentioned) the red cells rose from 1,750,000 to 2,450,000 and the leukocytes from 4500 to 9600 in forty days, while the hemoglobin made a gain of only three points during the same period; the nucleated red cells disappeared. The excessive administration of thyroid extract, on the other hand, causes a drop in the number of the red cells. (Regarding the results of removal of the thyroid gland see Cachexia Strumipriva.)

Serology.—In a number of instances of myxedema protective ferments causing the cleavage of thyroid tissue, have been demonstrated in the blood.

The Urine.—The total volume of urine is commonly diminished. A study of the nitrogen partition shows no essential deviation from the normal, and there is no evidence of any acidosis.

Albumin is found in about 20 per cent. of the cases and is apt to disappear when thyroid feeding is instituted.

NEPHRITIS (ACUTE)

Essential Factors.—Moderate secondary anemia; tendency to hyperleukocytosis. Albuminuria of mild grade with large numbers of hyaline, granular, and epithelial casts, renal epithelial cells and blood corpuscles (blood casts) in the tubular form. Marked oliguria; albuminuria of high grade; casts of all kinds; renal epithelium; red blood corpuscles and leukocytes in abundance—in the diffuse type. Bacteriuria; delayed phenolsulphonephthalein elimination; tendency to the formation of transudates.

The Blood.—*The Red Cells and Hemoglobin.*—If we bear in mind the numerous causative agents which can give rise to acute nephritis, it will be readily understood that the laboratory findings must differ very widely in different cases. When a nephritis develops in the course of an acute infectious disease the blood picture will naturally depend very materially upon the effect which such disease has upon the blood by itself. If this leads to anemia it may be very difficult to recognize the relative share which the nephritis is playing in its causation and extent. That the nephritis *per se* is at all responsible for the loss of red cells and hemoglobin may, indeed, be impossible to prove, but is rendered likely from a study of the toxic, exogenic cases (poisoning with mercury, glycerin, turpentine, carbolic acid, tar, chloroform, ether, etc.). Average figures are accordingly of no interest. Suffice it to say that in some cases of acute nephritis no anemia is demonstrable, while in others there is a rapid and extensive loss of corpuscles and of coloring matter. Generally speaking, this is proportionate to the intensity of the albuminuria, and according to

Hayem most marked in the hemorrhagic cases. As a general rule the anemia is moderate; in rare cases only does the loss of red cells exceed 2,000,000, and in all the oligochromemia is more marked than the oligocythemia.

The Leukocytes.—The same remarks which have been made above apply with equal force to the question of the occurrence of hyperleukocytosis. In those infectious cases in which an increased number of leukocytes existed already before the development of the nephritis, this complication may or may not give rise to a further increase. Regarding the findings in non-infectious cases our knowledge is unfortunately so meager that it is impossible to furnish any data of value. It appears, however, that here also the leukocytosis is variable. Generally speaking, hyperleukocytosis is the rule in acute nephritis; in Cabot's series of 50 cases values higher than 10,000 were found in 31. This writer attributes the increased counts to hemorrhage and uremia. In 12 cases in which these factors could be excluded Da Costa found the leukocytes above 10,000 in 9. In the infectious cases, no doubt, the leukocytic picture is essentially that of the underlying disease, but it would be interesting to see what the exogenic toxic cases would show.

The Plaques.—The plaques and fibrin formation are frequently increased.

Bacteriology.—In primarily non-infectious cases of acute nephritis the bacteriological examination of the blood is naturally negative, excepting *sub finem vitæ*, when a terminal infection may take place. In those cases, on the other hand, which develop secondarily to an acute bacterial disease the corresponding organism may, of course, be encountered.

The Urine.—The urinary picture in acute nephritis depends very largely upon the relative extent to which the glomerular portion of the kidneys is affected, and we may accordingly distinguish between tubular and diffuse cases.

A. Tubular Nephritis.—The mildest cases of this type are represented by the ordinary forms of febrile albuminuria, in which it is doubtful, in fact, whether one is entitled to assume the existence of an inflammatory process. The amount of albumin is usually slight, and on microscopic examination one finds only a few isolated hyaline casts. The urine otherwise presents the usual features, which are seen in acute febrile diseases, *i. e.*, it is high colored, diminished in amount, of increased specific gravity, and strongly acid.

Between these light cases and those in which an actual nephritis unquestionably exists there are all gradations, but even then (unless indeed hematuria complicates the case) the quantity of albumin is almost always small This is in marked contrast to the abundant sediment which is commonly observed. Microscopic examination reveals the presence of large numbers of renal epithelial cells (des-

quamative nephritis) in various stages of degeneration, hyaline, finely granular, and epithelial casts in variable number, a few leukocytes (unless the disease has extended to the pelvis, when they are numerous), and at times uric acid and oxalate of lime crystals. In the severe forms red cells also appear in variable number, occurring either as such or attached to casts (blood casts), some with their full content of coloring matter, others as mere shadows. Besides, there is always a considerable quantity of detritus, representing degenerated cells, amorphous pigment, etc.

Otherwise the urine shows the common features of a febrile process, its color is darker than normal, often it is of a reddish-brown color, and it is usually turbid. Chemical examination reveals the presence of nucleo-albumin (referable to disintegration of cellular elements), besides the common albumins of the serum, and in the hematuric cases of blood coloring matter.

B. Diffuse Nephritis.—In the diffuse cases (of which the scarlatinal form serves as a classical example) the urinary picture is correspondingly severe. There is marked oliguria from the start, which often, indeed, is the first symptom to attract attention. The amount may not exceed 100 c.c., and in especially severe cases there is an initial anuria. The specific gravity and color are correspondingly high (1.030 or more). In milder cases the oliguria is less extreme (300 to 600 c.c.) and the gravity normal. As blood is frequently present in microscopic amount the color is apt to be smoky or even a distinct meat-water red. In other cases it can only be demonstrated by the microscope. In still others in which hemoglobin is present in the free state it is a dirty brown or brownish red. In exceptional cases only is blood absent altogether.

Albumin.—Albumin is almost always present in large amounts, varying between a few pro milles and 1 per cent., or even more. As a considerable portion of this is serum globulin, the albuminous quotient of the urine is low. With improvement it rises and with exacerbations it falls. Nucleo-albumin is always demonstrable when the sediment is rich in cells. At times albumoses have been found, in the temporary absence of albumins, which may account for the occasional reports of scarlatinal nephritis urines that were free from heat-coagulable albumins. Blood-coloring matter is almost always present and tends to increase the albuminous content.

Microscopic examination shows the presence of large numbers of hyaline, epithelial, and coarsely granular casts, and at times also of blood and leukocytic casts. Generally speaking, their number is proportionate to the amount of albumin, but there are many exceptions. Sometimes there is very little albumin, while the urine is full of casts. In addition there are renal epithelial cells, occurring either singly or in masses and sometimes showing evidence of fatty degeneration; further, leukocytes, red cells, blood shadows, crystals of

uric acid or oxalate of lime, and in the hemorrhagic cases, blood-coloring matter in amorphous form. Besides these elements, bacteria may be met with, even though great care has been exercised to prevent the contamination of the urinary specimen from without. The inference hence suggests itself that the organisms in question may have been excreted through the kidneys, and *bacteriological examination* not infrequently shows that they are the same as those which cause the underlying disease. Pyogenic cocci are frequently found in corresponding infections. Von Jaksch states that in erysipelas the bacteriuria and nephritis disappear together with the cessation of the disease. In scarlatinal nephritis streptococci are found in a large percentage of the cases. In a series of 31 cases of nephritis collected at random, Engel found staphylococci in 16, streptococci in 8, the tubercle bacillus in 4, the colon bacillus in 5, and the typhoid bacillus in 1.

Other observers have met with the pneumococcus. The typhoid bacillus is found in probably every case of typhoid nephritis, and is frequently met with even though no inflammatory involvement of the kidneys exists. (See Typhoid Fever.) In bubonic nephritis the plague bacillus has been found.

General Metabolism.—A study of the general metabolism in acute nephritis shows a diminished elimination of nitrogen and especially of its chief exponent, urea, and a similar decrease of the chlorides and phosphates, while the output of uric acid is unchanged and that of the purin bases increased. Very interesting, further, is the impaired synthesis of hippuric acid, following the administration of benzoic acid. The elimination of various drugs, such as iodin, quinine, carbolic acid, methylene blue, etc, is impeded.

As *recovery* takes place the urinary picture gradually clears up. The amount of urine progressively increases, and polyuria may take the place of oliguria, especially during the resorption of transudates; the amount of sediment diminishes; the albuminuria and cylindruria become less and finally disappear; the casts sometimes persist for a while after the albumin is no longer demonstrable.

(For a consideration of special types of acute nephritis see the corresponding pathological conditions, *e. g.*, Cholera Asiatica.)

The permeation test with phenolsulphonephthalein shows delayed elimination which is the more marked the graver the clinical condition. The same is seen when an acute exacerbation occurs in the course of a chronic nephritis. In the five acute cases described by Rowntree and Geraghty the elimination of the first hour varied between 4.8 and 44 per cent. at the time of the first observation.

Transudates.—The formation of transudates is one of the most common symptoms of acute nephritis. Subcutaneous edema is usually the first to appear, but exudations into the serous cavities are likewise apt to occur at an early date. Sometimes there is a certain

parallelism between the degree of oliguria and the extent of the effusion, but this is by no means constant, and it is noteworthy, furthermore, that no relation exists between it, the intensity of the albuminuria, and the elimination of the various urinary components.

NEPHRITIS (CHRONIC)

Essential Factors.—Irregular chlorotic anemia; general tendency to hyperleukocytosis; diminution of the albuminous quotient of the blood; increased urea content of the blood. Oliguria, cylindruria, hematuria; marked albuminuria with increased albuminous quotient in the diffuse form. Tendency to polyuria, pollakiuria, and slight albuminuria with little or no cylindruria with increased albuminous quotient, in the indurative form. Delayed elimination with the phenolsulphonephthalein test.

The Blood.—*The Red Cells and Hemoglobin.*—While there is unquestionably a strong tendency to anemia in probably all forms of chronic nephritis, the actual findings differ very much in different cases; they depend to a great extent upon the stage of the disease, upon the nature of the underlying malady (when the nephritis is secondary), the existence of complications, etc. Not infrequently the actual anemia is obscured, more or less (so far as the laboratory findings are concerned and in contrast to the clinical condition), by the existence of a relative polycythemia, owing to a concentration of the blood in consequence of vomiting and diarrhea, the existence of cyanosis, etc. Average figures are hence of little interest. In the Hopkins series of 103 cases, mentioned by Emerson, the count was higher than 5,000,000 in 19, between 3,000,000 and 4,000,000 in 25, between 2,000,000 and 3,000,000 in 13, and below 2,000,000 (1,700,000) in 1. The hemoglobin in a series of 99 cases was above 80 per cent. in 17, between 30 and 50 in 29, and between 20 and 30 in 3. The anemia is thus manifestly of the chlorotic type. Generally speaking, it develops earlier and becomes more extensive in the chronic parenchymatous than in the chronic interstitial form. Exceptionally, cases are seen in which the intensity of the anemia approaches the numerical findings in pernicious anemia. McCrae has reported such a case, where the red cells numbered only 1,400,000, with 27 per cent. of hemoglobin. Cabot mentions another instance with a count of 1,468,000 and 23 per cent. of hemoglobin, and in Labbé's case the count had dropped to 500,000. In Cabot's case the diagnosis during the life of the patient was rendered especially difficult owing to the presence of both normoblasts and megaloblasts, while the red cells showed marked variations in size, poikilocytosis, and a tendency to oval form, and on one occasion 6 per cent. of myelocytes were counted.

In some instances of this kind it may be impossible to decide

whether the patient has only a nephritis or a nephritis complicating pernicious anemia.

Very low counts and still lower hemoglobin values may be met with in those cases in which the nephritis develops on the basis of a chronic lead intoxication.

The Leukocytes.—The leukocytes are increased in a large percentage of cases, both of the parenchymatous and the interstitial form. The cause of this is not always apparent, but sometimes attributed to an underlying or a complicating condition, while in others the impression is gained, as though the renal disease *per se* were directly concerned in its production. When uremia supervenes the tendency to hyperleukocytosis is slightly increased. This is not always manifest, if we compare the percentage of uremic with that of the non-uremic cases, for in Cabot's series of 92 cases of the latter type there was hyperleukocytosis in 66.3 per cent., while of the 94 uremic cases only 58.5 per cent. showed an increase. The average count, however, was higher in the first than in the last, viz., 15,100 as contrasted with 11,600. Cabot's highest count in the non-uremic series was 39,000, and 44,000 in the uremic (an eclamptic case).

So far as the differential count is concerned it appears that the hyperleukocytosis is always of the neutrophilic type, while the eosinophiles are diminished or disappear. Pieraccini concludes that the latter are diminished in proportion to the degree of toxemia; when this is severe they disappear; with a remission of the toxic symptoms they tend to return to normal or may exceed the normal; if, however, the toxemia becomes chronic the eosinophiles are not always diminished.

The Fibrin.—The fibrin, according to Hayem, is apt to show a greater increase in the chronic interstitial than in the parenchymatous form.

The Non-coagulable Nitrogen.—In 10 patients of advanced chronic nephritis, reported by Thayer, the non-coagulable nitrogen varied between 0.4 and 2 gm. per liter, the time of examination ranging between a week and fourteen months preceding death.

General Factors.—In the chronic parenchymatous form the hydremic condition of the blood is often manifest on naked-eye examination. The specific gravity is correspondingly low, but varies considerably in different cases and at different stages of the disease (1.026 to 1.062); the drop affects the serum especially (1.019 to 1.029; average, 1.023). Quite peculiar is the milky appearance of the serum, which has been variously ascribed to fat and albumin. Regarding the alkalinity of the blood there are no reliable data, as none of the older methods furnish accurate results.

Chemical Examination.—According to v. Jaksch the albuminous content of the blood serum in chronic parenchymatous nephritis is inversely proportionate to the degree of hydremia. Lecorché and

Talamon found that the loss in albumin affected the serum albumin to a greater extent than the globulin, so that the albuminous quotient $\left(\frac{\text{serum albumin}}{\text{globulin}}\right)$ is diminished, viz., 0.54 to 1.06 as contrasted with the normal, 1.5 to 2.

The urea content of the blood is constantly increased, even though no uremic symptoms exist, though the largest values have been found when these supervene. The increase may amount to ten to twenty times the normal value. The largest value met with by Babington was 1.5 per cent.; this occurred during a uremic attack complicating chronic interstitial nephritis, in which the patient died. The amount was the same in the urine. According to Widal the presence of from 1 to 2 grams per liter of serum usually indicates that the patient will not live more than a year; 2 to 3 grams that the span of life will not exceed many weeks or months, while a larger quantity than 3 grams indicates imminent peril.

The amount of uric acid and the percentage content of chlorine and sodium have been found increased, while potassium, iron, and phosphorus were diminished.

The Urine.—The urinary picture differs in the two essential types of chronic nephritis, *i. e.*, in the chronic diffuse, non-indurative (chronic parenchymatous), as contrasted with the chronic indurative type (chronic interstitial nephritis and renal sclerosis).

1. Chronic Diffuse Nephritis (large white kidney).—Oliguria is the rule in all cases, so long as the disease is active, and is, generally speaking, proportionate to the extent of the edema and associated transudation into the serous cavities. *Sub finem vitæ* it may become as extensive as in acute nephritis, but this is exceptional. At the height of the disease 250 to 500 c.c. are common values. With improvement the quantity rises, and rapid resorption of effusions may bring the amount up to several liters. The same occurs when the patient is caused to drink large amounts of water. In transitional cases (large mottled kidney) the urine is more abundant and gradually assumes the features which characterize the chronic interstitial form. The specific gravity is inversely proportionate to the amount of urine, and hence usually increased; sometimes with a very small quantity of urine it is very high—1.040 or more. The reaction is faintly acid and rapidly turns to neutral or alkaline on standing; at times it may be alkaline when voided. The color in the absence of macroscopically visible blood varies from a pale greenish yellow to a dark amber, depending essentially upon the degree of oliguria. On standing, the urine commonly deposits an abundant sediment, containing large numbers of hyaline, granular, fatty, waxy, and epithelial casts, renal epithelial cells undergoing extensive fatty degeneration, free fat globules, leukocytes, and red corpuscles, which are less numerous, however, than in the acute form, unless, indeed, a

special tendency to bleeding (hemorrhagic form) complicates the case, or unless an acute exacerbation supervenes. Generally speaking, the blood content is greater in the mottled than in the large white kidney, while the evidence of fatty degeneration is greater in the latter; to this rule, however, there are many exceptions.

Albumin.—Albumin is always present, and usually in greater amount than in the majority of the acute cases. It is roughly proportionate to the extent of the oliguria and the height of the specific gravity. Usually it varies between several pro milles and 1 per cent. In cases extending over many months the average daily elimination amounts to from 4 to 8 grams. Larger quantities are exceptional; Bartels has reported cases of this kind in which the albuminuria rose to from 4 to 6 per cent. As indurative processes come to the foreground in long-continued cases the albuminous content diminishes. The albuminous quotient is variable; usually the amount of globulin is small, so that the quotient is correspondingly high (2.09 to 5.48). Nucleo-albumin is either absent or present in minimal amounts, unless acute exacerbations occur with desquamation of large numbers of cells and their disintegration. Albumoses have at times been observed, but nothing is known of their origin.

General Metabolism.—As regards the general metabolism the urea values are usually lower than normal and particularly so in hydropic individuals. There are great variations, however, for which it is not always easy to account; some of these, no doubt, are referable to variations in the patient's appetite, the resorption of food material, the loss of albumin in the urine, while others may be due to temporary retention. The elimination of ammonia is normal, as is that of uric acid. The extractives (xanthin bases, kreatinin) are sometimes increased in connection with the rapid development of effusions or corresponding exacerbations. The excretion of mineral salts is variable; the chloride curve is roughly parallel to that of urea, and does not always correspond to the intake.

As in acute nephritis there is an insufficiency as regards the synthetic formation of hippuric acid and the elimination of various drugs.

The phenolsulphonephthalein test (which see) indicates the renal insufficiency particularly well. In the series of 24 cases reported by Rowntree and Geraghty the elimination of the first hour varied between 2 and 54.9 per cent., the greatest delay *cæteris paribus* being met with in the gravest cases.

The urinary picture in chronic diffuse nephritis thus closely resembles that of the acute form, but it must be borne in mind that the pathological anatomical progress of the disease is usually marked by remissions and exacerbations, which lead to analogous changes in the condition of the urine. As acute exacerbations occurring in interstitial nephritis produce a similar urinary condition, it will readily be understood that the diagnosis of chronic diffuse nephritis

may be exceedingly difficult. In youthful individuals this is less so, but in older persons it may be impossible

2. Chronic Indurative Nephritis (atrophic kidney); chronic interstitial nephritis; arteriosclerotic kidney

In the early stages of chronic indurative nephritis there may be no urinary changes whatever to attract attention, and in some cases of arteriosclerotic kidney the patient dies without having shown albumin in his urine. In others, and particularly in those cases of chronic interstitial nephritis which develop without any apparent cause in relatively youthful individuals, there is for years an irregular albuminuria which not infrequently is viewed as physiological and commonly neglected. In still others the condition is preceded by a chronic diffuse nephritis and the corresponding urinary picture. Often the development of pollakiuria (frequent micturition, at first attracting attention at night) or a polyuria are the first symptoms which lead the individual to seek medical advice. In definitely developed cases the polyuria is one of the most constant symptoms. Usually the amount varies between 2000 and 3000 c.c.; sometimes it falls temporarily to normal, or may even become subnormal, while at other times it increases to 4000 to 5000 c.c. Higher values are exceptional. Lecorché and Talaman speak of a case where the amount reached 10 liters in the twenty-four hours. The urine is pale and does not even darken to any great extent when, as a result of a complicating febrile process, the amount is temporarily diminished. It is clear or slightly turbid owing to bacterial development which takes place with great readiness, unless some preservative has been added. The reaction is feebly acid and the specific gravity diminished; usually this is about 1.010; rarely it drops below 1.005.

In well-developed cases albumin is usually present, often continuously so, but not infrequently intermittently, such that the morning urine is free while in the evening albuminuria occurs; sometimes a urine which is ordinarily non-albuminous readily becomes albuminous following a liberal meal. Fluctuations are thus exceedingly common. The amount, however, barring periods of acute exacerbation, is almost always small; frequently one can only speak of traces and even at the height of the disease it rarely exceeds 0.5 pro mille. The daily loss usually does not exceed a few grams; 10 grams is rarely met with. Albumin and globulin are both present, the latter usually in small amount, so that the quotient $\left(\frac{\text{serum albumin}}{\text{serum globulin}}\right)$ is high (1.3 to 3.0).

Corresponding to the low specific gravity there is very little, if any sediment. A microscopic examination is, indeed, often possible only by centrifugalizing a larger amount. In this manner enough material may be obtained to establish the presence of a small number of hyaline or finely granular casts, an occasional epithelial cell, of which the origin can frequently not be ascertained, a few leukocytes, occa-

sionally uric acid or oxalate crystals and exceptionally only a small number of red corpuscles. On rare occasions, more particularly after overexertion, actual hematuria may be observed.

General Metabolism.—A study of the general metabolism shows a normal absolute elimination of urea, while the relative values, corresponding to the polyuria, are, of course, low. The same holds good for the excretion of ammonia and of uric acid, while the output of purin bases is said to be increased. Such is the picture in the majority of cases so long as no serious symptoms supervene. With the development of uremic symptoms the ratio of urea to the total nitrogen falls (as low as 58 per cent.), while the ammonia values frequently, though not invariably, rise. Simultaneously the uric acid value falls and the purin output increases. The chloride curve generally follows that of the urea. The same usually holds good for the sulphates, but at times there is a decrease; this seems to be constant for the phosphatic elimination, both relatively and absolutely.

The elimination of various drugs, in contradistinction to the chronic diffuse form, is frequently not disturbed, but the *phenolsuphonephthalein test* (which see) usually (possibly always) indicates the existence of renal insufficiency. Rowntree and Geraghty recite a number of very instructive cases in which the diagnosis from the clinical symptoms shown could not have been made, but in which the permeation test indicated the true state of affairs as proven by the subsequent course of the malady. The lowest values, as one would expect, were met with in the uremic cases (see Uremia). In a series of twenty cases of advanced chronic nephritis with more or less granular atrophy in gross, in which careful postmortem examinations were made, Thayer and Snowden found an excretion (in two hours) varying from 0 to 38 per cent. in 11 cases which were examined within a week before death. In 7 patients of the same order in which the test was made within a month or over a week before death, the excretion varied from a trace to 34 per cent., and in 5 additional patients in which the examination was made at periods of from one to eight months it ranged between 1.5 and 35 per cent. In 7 further cases of chronic nephritis in which the autopsy showed this to be of moderate extent the values ranged from 16 to 42 per cent., the time of examination ranging from one day to eighteen months before death.

In 20 cases of chronic passive congestion (all of which clinically were examples of cardiac dilatation and insufficiency), the values ranged between a trace and 71 per cent. and the time before death between one day and eight and a half months.

Thayer and Snowden summarize their observations as follows: "In severe chronic nephritis the pthalein output is uniformly low and decreases steadily up to the onset of uremia and is nearly or wholly suppressed from a day or two to a month before death,

excepting in cases which are not interrupted by an acute terminal process. Acute processes, however, which may be unsuspected clinically, are common, and here a sudden diminution in the elimination, may come on in cases where the percentage previously excreted was not so low as to appear menacing.

Chronic passive congestion (cardiac disease) results often in a considerable reduction in the two hours' elimination. The results are very variable in individual cases. In marked decompensation the output may be reduced to but a trace in two hours; but the excretion is, as a rule, rapidly restored with the reëstablishment of circulatory compensation.

When acute exacerbations supervene, when uremia develops, or when stasis occurs, the urinary picture, as above outlined, undergoes changes approaching the parenchymatous form (which see).

Cryoscopic examination of the urine may well be dispensed with in the diagnosis of nephritis.

Transudates.—Transudates of any moment are usually only observed, *sub finem vitæ,* when cardiac compensation has been definitely broken. At other times there are no effusions whatever, or at most slight edema over the tibiæ, about the ankles, or in the morning hours about the eyelids.

NEPHRITIS (SUPPURATIVE, AND RENAL ABSCESS)

Essential Factors.—Secondary anemia; neutrophilic hyperleukocytosis; irregular bacteriemia; hematuria; pyuria; occasional presence of bits of necrotic tissue.

The Blood.—*The Red Cells and Hemoglobin.*—As all suppurative processes in which absorption is taking place lead to anemia, it will readily be understood that a suppurative lesion involving the kidneys, irrespective of any underlying pathological condition (ulcerative endocarditis, pyelitis, paranephritis, psoitis, peripsoitis, etc.) will sooner or later in itself cause the destruction of red cells, with a consequent loss of hemoglobin. The degree of anemia will largely depend upon the duration of the malady and the intensity of the infection.

The Leukocytes.—The leukocytes are increased in all cases, the leukocytosis being of the neutrophilic type, associated with a decrease or absence of the eosinophiles. In short, there is the typical picture of a septicemia, and on bacteriological examination it may be possible to demonstrate the offending organism in the general circulation; in many cases, however, the cultures are sterile. (See Septicemia.)

The Urine.—The urinary picture is variable. In cases which develop from traumatism, hematuria or anuria are sometimes the first symptoms to attract attention, which may then be followed after a variable length of time by a discharge of pus. In those cases in which a suppurative nephritis develops by contiguity the diagnosis

may be excessively difficult. The appearance of pus *per se* does not indicate that the kidney structure proper has been involved, as a perinephritic abscess may rupture directly into the urinary tract. If, however, the discharge of pus is associated with the disappearance of a corresponding mass or a diminution in its size the inference is justifiable that the kidney structure itself was involved. While pyuria is the rule in these two types of suppurative nephritis, it may occur that the pus is encapsulated and cannot be eliminated. As the disease is almost always unilateral in these cases, it may accordingly happen that a normal urine is passed. This presupposes, of course, that the other kidney is intact.

The reaction of the urine in suppurative nephritis may be acid, neutral, or alkaline. Frequently ammoniacal decomposition takes place already within the urinary passages. The sediment, in addition to pus and blood, then contains triple phosphates and ammonium urate crystals. On rare occasions, bits of renal tissue may be found. Albumin is either absent or present in traces. If an ordinary non-suppurative nephritis complicates the condition, a corresponding albuminuria and cylindruria will be observed.

When the condition has developed as an extension of a pyelitis, the urinary picture will be accordingly modified. (See Pyelitis.)

Metastatic renal abscesses, owing to their small size, usually escape recognition. The condition may be suspected if early in the septicemic condition there occurs a very notable diminution in the secretion of urine or complete anuria. This may be an indication that the glomeruli and tubules have been obstructed by bacteria.

NEURASTHENIA

Essential Factors.—There are no constant factors.

Considering the fact that the term neurasthenia is essentially symptomatic, it seems scarcely warrantable to attempt the production of a collective picture of the laboratory findings in what unquestionably is not a pathological entity. For practical purposes, however, it may not be out of place.

The Blood.—*The Red Cells and Hemoglobin.*—In some neurasthenic individuals the red count and hemoglobin values are absolutely normal, but in many others there is unquestionably a definite tendency to a mild grade of chlorotic anemia. Sometimes this is partly obscured by a moderate concentration of the blood. This is particularly noticeable in thin, dyspeptic patients, where there may be a marked discrepancy between the apparent anemia and the actual findings. The common saying that not every pale face indicates the existence of anemia would seem particularly applicable in such cases, but it may be added that the color of the face is often

a better index of existing conditions than the hemoglobin value and especially the red count.

The Leukocytes.—The leukocyte count and the differential findings are normal; any abnormality in this respect may be referred to some complicating condition.

The Stomach Contents.—In some neurasthenic individuals, analysis of the gastric juice shows a perfectly normal condition, but in others there are more or less marked abnormalities. Most frequently one meets with various grades of hyperchlorhydria, less commonly with hypochlorhydria and anachlorhydria; in some there are more or less abrupt changes from the one condition to the other. The majority of cases of so-called *Reichmann's disease, hypersecretio acida et continua* (which see), unquestionably are neurasthenics.

The ferments are far less apt to be affected by the neurotic process. For chymosin Boas has demonstrated that even in the absence of free hydrochloric acid the zymogen may still be present in normal amounts, *i. e.*, demonstrable with a dilution of 1 to 100 to 1 to 150. For pepsin (sc. pepsinogen) the same holds good. Lactic acid is always absent, even though no free hydrochloric acid be present.

Under the term *gastrosuccorrhea mucosa,* Dauber has described a condition in which large amounts of mucus are secreted by the non-digesting organ, in the absence of symptoms pointing to a gastritis. I have observed a similar case in a neurasthenic patient, in whom enormous quantities of mucus could at times be obtained from the fasting organ, but never during the process of digestion. A mild degree of hyperchlorhydria existed at the same time, as well as enteritis mucosa and rhinitis mucosa. The motor power was practically normal.

Motor insufficiency of variable degree is likewise a common factor in neurasthenic individuals, and in exceptional cases actual dilatation may develop upon such a basis.

The Feces.—As in the case of the gastric contents, so here also the findings may be quite variable. In many cases, notably those associated with hyperchlorhydria, there is a tendency to stubborn constipation, and at stool hard scybalous masses are frequently passed. In others there is diarrhea of variable degree, and apparently without cause; in some of these all the movements are thin, while in others diarrhea only occurs in the morning, and normal stools may be passed later in the day. In all such cases, a careful microscopic examination of the stool is indicated, so that an amebic colitis or a trichomonas infection is not neglected and passed over as "simply neurotic."

Very common is the passage of *mucus.* In some instances this may be referable to an associated "mucous colitis" (which see), while in others there is no ground for the assumption of a definite

organic basis. The amount in these neurotic cases is more often large than small, and at times a whole cupful may be passed in the absence of fecal material.

The Urine.—The urinary picture presents no features which can in any way be regarded as characteristic. In some cases one meets with polyuria, in others with oliguria; in still others the two alternate, while again in others the flow is perfectly normal. The output of mineral salts is essentially dependent upon the patient's appetite; when the amount of food ingested is normal, the chlorides, phosphates, and sulphates also will be found in normal quantities. The same is true of the excretion of urea and of uric acid, and I would emphasize that the clinical diagnosis of uric acid intoxication in neurasthenic individuals is almost invariably without any adequate chemical basis. Of special interest, however, is an acid intoxication which is noted in isolated cases, and to which Friedrich Müller has drawn especial attention. These cases are characterized clinically by general neurasthenic symptoms and special symptoms referable to the digestive organs. The urine is somewhat concentrated, with relatively high specific gravity, markedly acid, and on standing deposits a fairly abundant sediment of either uric acid crystals or of calcium oxalate, or both. The total acidity values are high, and on deducting the acid phosphates there is a markedly increased acid remnant which must be attributed to organic acids; what these acids are, however, is not known.

Albumin is usually absent, but in some neurasthenics, especially in anemic young men with tendencies to masturbation, traces are not infrequently encountered, which are possibly of prostatic origin. In these cases one also finds spermatoza almost at all times. Renal albuminuria, when it actually occurs, has probably nothing to do with the neurasthenic state.

Glucosuria, while not a feature of the disease in itself, can apparently be brought about artificially in some of the cases. Strauss emphasizes that whereas glucosuria is rarely observed in organic lesions of the spinal cord, following the administration of 100 grams of glucose, it is not uncommon in the *traumatic neuroses*. He observed this phenomenon in 37.5 per cent. of his 40 cases, while in the non-traumatic cases only 14.4 showed an insufficiency in this respect.

Indicanuria, at times of considerable intensity, is observed in some gastro-intestinal neurasthenics, and persists, in spite of treatment, for months and even years.

The microscopic examination reveals no special points of interest beyond those already mentioned; tube casts in particular are only seen in those exceptional cases where so-called functional albuminuria complicates the condition; even then they are frequently absent, or if present they are found in very small numbers, the hyaline casts being almost exclusively present.

NEURITIS

The Blood.—Cabot mentions 9 cases of *febrile multiple neuritis*, apparently of infectious character, in which blood examinations were made. In 2 of these the leukocyte count ranged between 16,000 and 28,700; in 4 others between 10,100 and 10,900, while it was practically normal (6400 to 9600) in the remaining 3. Differential findings are given in only 1 (total count, 8400), viz., lymphocytes, 62.0; polynuclear neutrophiles, 36; and eosinophiles, 2 per cent.

The hemoglobin values range from 42 to 85 per cent. Red counts are mentioned in only one, viz., 4,320,000 to 4,816,000.

In 4 *alcoholic afebrile* cases the leukocytes varied from 6700 to 21,300, the red cells from 3,260,000 to 3,608,000, and the hemoglobin from 45 to 90 per cent.

In 1 case of *postdiphtheritic neuritis* the red count was 3,850,000, the white 7393, and the hemoglobin 70 per cent.

(Regarding the findings in neuritis due to lead, see the section on Lead Poisoning.)

The Urine.—The urine has not received special study in neuritic cases, but the findings will, no doubt, depend upon the nature of the underlying malady. In the majority of cases the condition will probably be found normal, while in some instances slight albuminuria may be anticipated.

OBESITY

Essential Factors.—Irregular tendency to polycythemia and high hemoglobin values; lymphocytosis; irregular tendency to glucosuria; albuminuria in advanced cases.

The Blood.—*The Red Cells and Hemoglobin.*—The majority of obese individuals have a normal or slightly increased red count with corresponding *hemoglobin* values. In 79 of the 100 cases recorded by Kisch the hemoglobin exceeded 100, with 120 as maximal figure. Exceptionally there is absolute polycythemia, as in 2 cases recorded by v. Noorden, where the counts were 7,200,000 and 7,700,000 respectively. In a smaller percentage of cases (21 per cent. of Kisch's series) there is moderate anemia, which, however, is referable only in part to the obesity, but rather to complicating conditions, such as syphilis, alcoholism, menstrual disturbances, etc. Late in the disease, when weak heart and edema exist, the hemoglobin values may fall to one-half of the normal, while the red cells are affected to a less extent.

The Leukocytes.—According to Kars there is a constant lymphocytosis with a tendency to maximal normal total values. Under the influence of thyroid tablets the lymphocytosis decreases or returns to normal ratios with a corresponding rise of the polynuclear neutrophiles.

Fat.—The statement has been made repeatedly that the blood of particularly obese individuals contains an increased amount of fat, as compared with the normal. Kisch thus states that the amount may rise from 0.2 to 0.3 to double and treble this amount. Further studies in this direction, however, are required. I have never seen the milky turbidity in such cases which one meets with in advanced diabetes, unless the examination was made during the process of digestion, when similar findings are obtained in normal individuals.

The Urine.—In cases which are uncomplicated by renal or cardiac disease the daily elimination of urine is normal, or, when free perspiration has taken place, somewhat diminished. Kisch's average of 25 cases was 1450 c.c. Von Noorden found variations between 1250 and 1550 c.c. in men, and 1080 to 1350 c.c. in women. Upon reducing the intake of water there is a corresponding reduction in the output, which then generally corresponds to 68 to 80 per cent. of the amount ingested. If weak heart and edema complicate the case, this reduction in the secretion of water does not always occur to the same degree; on the contrary there is frequently no change in the amount, but even an increase (Oertel).

Nitrogen Partition.—The nitrogen partition is normal, viz., 85 to 88 per cent. of urea-N., 3 to 6 per cent. of ammonia-N., and 1 to 2 per cent. of uric acid-N. There is often a marked tendency toward the deposition of calcium oxalate crystals in the urine of the obese, but there is no actual increase in the elimination. Kisch's figures vary between 4.9 and 18 mg. per liter; in only 1 case was a larger amount noted, viz., 40 mg.

In the hypophyseal form of the disease the endogenous *uric acid* output is said to be normal, while the administration of sodium nucleinate does not cause a material increase in the elimination of the substance (see also Acromegaly).

Acetonuria.—Acetonuria (aside from normal traces) does not occur, even though the patients are placed on a diet in which the carbohydrates have been materially reduced for a long time. If, however, carbohydrates are ingested in large amounts at first, and are then suddenly withdrawn, the acetone curve rises abruptly, as in normal individuals. When obese patients are placed on starvation, however, not only acetone, but also diacetic acid and especially oxybutyric acid appear in considerable amount. Very curiously the acidosis is greatest during the first starving period, while on subsequent occasions, which are separated from the first by an interval of ten days or a fortnight, it is less extensive.

Glucosuria.—Digestive glucosuria (following the administration of 100 grams of glucose) is not an uncommon event in the obese, and may, according to v. Noorden, be the forerunner of *diabetes.* Regarding the frequent coincidence of diabetes and obesity the same writer suggests that in some of these cases the diabetes may be primary and the excessive accumulation of fat secondary.

Albuminuria.—Albuminuria occurs in about 26 to 27 per cent. of all cases of obesity, and is especially apt to develop in extreme cases and those of long duration, being no doubt referable to circulatory abnormalities and actual nephritis.

OROYA FEVER

The Blood.—In all cases there is a more or less severe anemia, which in severe cases develops with great rapidity and may assume a pernicious type. The Harvard expedition reported one case with a red count below 1,000,000 and cite a count by Monge of 500,000. The hemoglobin values may fall as low as 15 per cent. in severe cases, while in others they vary between 40 and 50 per cent. Nucleated red cells appear early and rapidly increase in number; the majority are normoblasts, but megaloblasts also are frequently seen. Polychromatic and stipple cells are numerous and both anisocytosis and poikilocytosis are marked. Blood crises are apparently common in uncomplicated cases.

Hyperleukocytosis is quite constant, counts of 25,000 being common, while the differential shows no special deviation from the normal to account for the high total counts, which suggests that the latter only represent a relative increase and no increased production of any one kind. The eosinophiles vary between 0 and 10 per cent. (!)

In severe cases the corresponding parasites (which see) are found in the red cells in large numbers, while in mild cases the search for them may be quite tedious.

The Urine.—There may be a trace of albumin.

OSTEOMALACIA

The Blood.—*The Red Cells and Hemoglobin.*—The red cells and hemoglobin are usually somewhat diminished (with lowered color index), but normal values are also quite common.

The Leukocytes.—The leukocytes may be normal, diminished or slightly increased. There is commonly a lymphocytosis (up to 56 per cent.), with a moderate increase of eosinophiles (10 per cent.) and the presence of a few myelocytes. These changes, however, are apparently not constant.

The alkalinity of the blood shows no essential changes.

The Urine.—Examination of the urine shows a loss of calcium, associated with a retention of nitrogen, sulphur, and magnesium.

OSTEOMYELITIS.

The Blood.—*The Leukocytes.*—In osteomyelitis, in contradistinction to tuberculosis of the bones, a hyperleukocytosis due to an increase

of the polynuclear neutrophiles is seen in the acute and subacute cases, the average count in the acute stage being about 22,000, as compared with 11,400 in tuberculosis (Fiske).

OTITIS MEDIA AND MASTOIDITIS

Essential Factors.—Hyperleukocytosis with septic factor; negative bacteriological blood findings, excepting in cases of complicating sinus thrombosis and meningitis. Predominance of streptococci in the purulent exudate from the ear.

The Blood.—*The Red Cells and Hemoglobin.*—The red cells and hemoglobin are but little affected in median otitis and mastoiditis, unless the infection has persisted for some length of time, in which case secondary anemia, of greater or less intensity, is observed.

The Leukocytes.—The leukocytes are increased in almost all cases. In the serous form of otitis the hyperleukocytosis is usually slight (10,000 to 17,000) and occasionally absent, while in the purulent cases a brisk increase is the rule (up to 25,000 and even 30,000). The same holds good for mastoiditis and complicating intracranial suppuration. In all such cases the differential count will reveal the septic factor. The increase of the neutrophiles may vary from 75 to 98 per cent., and is, generally speaking, proportionate to the intensity of the infection and irrespective of the total number. The same considerations, in fact, which apply to septic processes in general also apply here.

The Bacteriology of the Blood.—In a series of 75 cases of otitis media or mastoid disease without sinus thrombosis or meningitis, Libman and his collaborators found the blood sterile in all. Positive results were obtained only in cases presenting the latter complications (including thrombosis of the jugular bulb). In 30 cases of this order Libman found a bacteriemia in 23 (77 per cent.); in 20 of these streptococci were isolated, in 2 the Streptococcus mucosus, and in 1 the Bacillus proteus; the pneumococcus was not encountered in any of the cases.

Libman's studies in this direction are so important that I have taken the liberty to quote him verbally *in extenso:*

"Our studies show that cases in which streptococci are found in the blood after the disease of the mastoid is properly operated upon, and in which clinical symptoms persist and no other cause can be found for a streptococcemia, are cases of sinus thrombosis. It is very important to consider carefully the possibility of other causes of streptococcemia (or other bacteriemia) in such cases. In our investigations the cases of otitic disease which even before operation showed a streptococcemia, and in which other causes for a streptococcemia could be excluded, showed sinus thrombosis at operation or at autopsy. We would not be willing to state absolutely, however, that in all

the cases of the latter group a sinus thrombosis would be found, because our studies in cases of acute otitis media and mastoiditis uncomplicated by sinus thrombosis are not extensive enough. But if the middle ear has been properly drained, and the mastoid has been operated upon, and there is no meningitis present, and no other cause found to explain the persisting bacteriemia and persistent clinical symptoms, we come, practically by exclusion, to the assumption that a lesion is present in the veins which causes the bacteriemia.

"It is necessary, in studying such cases, to take a number of points into consideration. There is always a possibility that a patient is suffering from a general infection, in the course of which he develops otitis media or mastoid disease. This is particularly liable to occur in cases of general pneumococcic or streptococcic infection, cases of pneumonia[1] and cases of typhoid fever. In cases of typhoid fever it is essential to know whether or not the otitis media developed after the patient has already had fever. The presence of typhoid bacilli in the blood, if one were in doubt as to whether marked symptoms were due to an otitis media by itself or to a complicating typhoid, would make us feel that we could ascribe the fever to the typhoid infection alone. The rest of the diagnosis would have to be based on clinical signs and on the development, possibly, of a streptococcemia, although it is to be remembered that rarely a patient with typhoid fever suffers from mixed infection with streptococci. In some of these cases it might be very difficult to come to a conclusion as to the exact condition in the mastoid and surrounding parts.

"It is also important to note the possibility of a bacteriemia following operative interference upon the middle ear or the mastoid process. In all of our cases in which we found streptococci in the blood after the mastoid had been operated upon, a sinus thrombosis was found. But from our experience with postoperative bacteriemia after operations in other parts of the body, we believe that one should be very careful in judging of positive blood cultures, if the blood cultures are taken directly after there has been operative interference.

"It has often been pointed out that difficulties might arise because of the possibility of a developing erysipelas or the presence of an angina. It has been feared that one would have difficulty in interpreting a streptococcemia in a case of otitic disease, if either of these conditions was present. As a matter of fact, in our experience we have very rarely found streptococci in the blood in erysipelas, and then it has only been in fatal cases. This experience seems to be shared by other investigators of the blood in this disease.

"The question of a possible confusion because of the presence of an

[1] If in a case of otitis media due to streptococci, pneumococci should be found in the blood, one would have to think of a developing lobar pneumonia.

acute angina is a very important one. Streptococcemia in such cases is very infrequent. In our experience we have fortunately had no trouble from this source.

"Leutert and, later, his assistant, Nuerenberg, have suggested a method for attempting to overcome the difficulty involved under the conditions mentioned above. Leutert has suggested taking blood cultures from the sinus and from the jugular vein and an arm vein. He believed that if more streptococci were found in the blood of the sinus than in the blood of the jugular vein or an arm vein, or if organisms were found in the blood of the sinus and none in the blood of the peripheral vein, one could conclude that the bacteria were gaining entrance to the circulation from the sinus, and that the bacteria were not entering the blood from the coincidental angina or erysipelas. They describe several cases in which they found this method valuable, but they admit what at once must strike any one as a serious objection. The results cannot always be depended upon, because the sinus is aspirated through an infected area. In one case we aspirated the sinus because we could not obtain blood from one of the peripheral veins. The blood from the sinus was sterile; a clot found at operation was also sterile.

"In our cases the number of bacteria found in the blood in cases of sinus thrombosis accompanied by bacteriemia has varied from less than 1 to the cubic centimeter up to 245 to the cubic centimeter. In all of the cases, except those that died within a few days after admission, we attempted to ascertain how rapidly the bacteria disappeared from the blood after operative interference. In all except 3 cases the bacteria disappeared rather promptly. As a rule, the bacteria did not disappear until the jugular vein was tied. There were 2 cases, however, in which the bacteriemia disappeared after a clot was removed from the lateral sinus and the jugular vein was not tied.

"In our early studies we took the secondary cultures forty-eight to seventy-two hours after operation, and we found that the bacteria had disappeared in all the cases except in the 3 mentioned. In some of the latter cases we took the blood cultures twenty-four hours after ligation of the jugular vein, and these cultures also gave a negative result. It is very remarkable to see as many as 245 streptococci to the cubic centimeter of blood disappear within twenty-four to forty-eight hours after ligation of the jugular vein. In 1 case there were 7 colonies of streptococci to the cubic centimeter of blood; within eight hours after ligation of the jugular vein the blood culture was negative and remained so.

"The three cases in which bacteria did not disappear promptly after ligation of the jugular vein were of particular interest. In one the streptococcus persisted in the blood after the clot had been

removed from the lateral sinus and the jugular vein had been tied. The bulb of the jugular vein was then exposed, a clot removed, and the bulb packed; the bacteriemia (there were 2 colonies to the cubic centimeter of blood) then promptly disappeared in less than four hours. In this case we believe the continuance of the bacteriemia was probably due to infection of the general system by way of the inferior petrosal sinus.

"In another case the bacteriemia disappeared after ligation of the jugular vein, but a couple of metastatic deposits developed. The patient also had a right-sided mastoiditis, which was operated upon. Later, streptococci were again found in the blood, the patient developed more metastases, and finally recovered. Although at the time we did not think of the possibility, we now believe that it is probable that in this case there was also a thrombosis on the right side, from which the patient recovered without operation. We know that some cases of sinus thrombosis with or without metastases have recovered without operation. The same holds true of infected veins in other parts of the body.

"In the third case, in which the bacteria persisted even after the local focus had been thoroughly dealt with (and this was confirmed at autopsy), the persistence of the bacteriemia was due to the development of an acute (ulcerative) endocarditis."

The Bacteriology of Otitis Media per se.—According to the studies of Libman and his collaborators, based upon observations of 277 cases, streptococci, either alone or associated with other organisms, were found in 189 cases (81.46 per cent.), streptococcus mucosus twenty-times (10.34 per cent.), and the pneumococcus nineteen times (8.2 per cent.). Libman points out that earlier investigators found the pneumococcus more frequently than the streptococcus, while more recent writers have noted the opposite. This apparent divergence of results is, no doubt, referable to more exact recent methods of differentiation between the organisms in question. Other organisms that were encountered are Staphylococcus aureus and albus, the Bacillus pyocyaneus and proteus, and exceptionally the diphtheria bacillus the colon bacillus, Gram-negative cocci, the xerosis bacillus, and others. Mixed infections were not uncommon, but in the majority of cases pure infections with the streptococcus were observed.

Abert's studies, which are based upon the examination of the surgically opened mastoid process instead of the middle ear by way of the external meatus, found the following: Of 110 cases over 90 per cent. showed streptococcus; in 18 cases streptococcus alone was present, in 19 almost only streptococci; while in 73 there was a mixed infection, streptococci, however, predominating; in two cases saprophytes only were found. In the middle ear infections following influenza no influenza bacilli were found.

PANCREATIC CYST

Essential Factors.—Irregular hyperleukocytosis, steatorrhea, azotorrhea, and glucosuria; clear or hemorrhagic fluid in the cyst, with presence of digestive ferments, notably of diastase.

The Blood.—In the absence of inflammatory complications the blood shows no material deviation from the normal, unless, as is occasionally the case, the cyst develops as a complication or sequel of acute hemorrhagic pancreatitis.

The Feces.—Steatorrhea and azotorrhea may be observed when the cyst is situated in the head of the pancreas (which is the more uncommon site), since in such cases the large duct may be occluded.

The Urine.—Diabetes is rarely observed in connection with pancreatic cysts—only in 9 of the 134 cases collected by Oser.

Digestive glucosuria has been observed in a single instance, by Lazarus. It may develop, however, as a late sequela after operation, in consequence of scar tissue formation and coincident destruction of islands of Langerhans.

Cystic Contents.—The contents of the cyst are sometimes clear and watery with a yellowish tint, while in other cases, and probably more frequently, they are hemorrhagic, the color, owing to a variable degree of tryptic digestion, varying from a brownish red to a coffee-color, occasionally with a greenish tone. Chemical examination may show the presence of trypsin at the time of operation, but more commonly a diastatic ferment only is demonstrable. It should be noted, moreover, that tryptic ferments may be found in cystic contents of other origin, and are hence, even if present, of no diagnostic significance. Even if all three ferments of the pancreas can be demonstrated the nature of the cyst is not necessarily proved.

Microscopic examination reveals the presence of red blood corpuscles in various stages of degeneration, a variable number of leukocytes, epithelial cells (at times), fat globules, fatty acid needles, and necrotic tissue.

Ascites.—Ascites occasionally develops in consequence of compression of the portal vein.

PANCREATIC LITHIASIS

The laboratory findings in pancreatic lithiasis are essentially those of pancreatitis, acute or chronic, according to the nature of the case, or they simulate those of pancreatic cancer (which see).

PANCREATITIS (ACUTA HÆMORRHAGICA)

Essential Factors.—Hyperleukocytosis of the neutrophilic type; a decrease in fecal diastase; absence of steatorrhea and usually of glucosuria.

The Blood.—*The Red Cells and Hemoglobin.*—The red cells and hemoglobin are frequently diminished in acute as well as in subacute pancreatitis. In the series of 4 cases recorded by Da Costa the lowest count was 1,550,000, and the highest, 4,460,000, with hemoglobin values ranging between 26 and 88 per cent.

The Leukocytes.—The leukocytes are increased in most cases. Early during the attack this is comparatively slight, however, with but little disturbance of the normal differential values. Later, when gangrene and infection have occurred, the neutrophiles rise and cause a more marked increase in the total number of the leukocytes. Taking the 7 cases of Da Costa and Cabot conjointly, there was leukocytosis exceeding 10,000 in 6, the highest figure being 32,000. Hunt mentions another case with 37,000.

The Feces.—At the onset of the disease there is frequently constipation, but in some cases, and usually in all cases where the stage of gangrene has been reached, there is diarrhea. In contradistinction to the chronic form of the disease, steatorrhea is rarely observed. The amount of fecal diastase is quite constantly diminished.

The Urine.—According to its originator the Cammidge reaction was announced as present in probably all cases, and an important factor in the diagnosis of the condition. This view, however, is not shared by the majority of subsequent investigators. Wilson, reporting from Mayo's clinic, thus refers to the reaction as "both valueless and misleading," and Kinney, from Deaver's clinic, remarks that little dependence can be placed upon a negative reaction and that a positive reaction can only be considered of confirmatory value.

Glucosuria may be observed, but is exceptional. In 3 cases seen by Cammidge it was absent. Benda and Stadelmann mention an instance where the symptoms were those of a rapidly fatal diabetic coma, with 3.4 per cent. of sugar. In some of the cases, however, in which glucosuria was noted, the patients had previously suffered from diabetes. The possible association of the two conditions should be borne in mind.

Lipase has been found in the urine of acute pancreatitis by Opie and Hewlett, while Pratt obtained negative results. Other observers (Wohlgemuth, Hirschberg) speak of the appearance of diastase in considerable amount.

Cyst Formation.—In some cases of acute pancreatitis pancreatic or peripancreatic cysts develop as a complication or sequela. Rasumowsky mentions a case of this order where the tumor appeared

five hours after the onset of the symptoms. At operation one finds necrotic pancreatic tissue and blood either as such or more or less digested by trypsin.

PANCREATITIS (CHRONICA)

Essential Factors.—Irregular blood changes; steatorrhea; azotorrhea; a decrease in fecal diastase; absence of glucosuria, excepting in advanced cases; choluria; presence of increased amount of diastase in the urine during acute exacerbations of the disease.

The Blood.—The blood picture in chronic pancreatitis depends upon the existence of associated pathological conditions (gallstones, alcoholism, etc.). In some of the cases there are no material deviations from the normal. In three cases mentioned by Da Costa the red cells ranged between 3,600,000 and 5,900,000, the hemoglobin from 54 to 99 per cent. and the leukocytes between 4000 and 11,300.

The Feces.—In the majority of cases of chronic pancreatitis the stools are bulky (see amount of feces, p. 210) and contain abnormally large amounts of fat (steatorrhea). It was once thought that this condition was pathognomonic of the disease in question. We now know, however, that the bile itself is capable of effecting the resorption of fat and that cases of extensive pancreatic degeneration may occur, on the one hand, in which no steatorrhea exists, while on the other, analogous findings may be obtained in the absence of pancreatic disease (chronic tubercular peritonitis, simple duodenal catarrh, amyloid disease of the intestinal mucosa). Biliary obstruction in itself can also give rise to steatorrhea, though it must be remembered that in most cases where this has persisted for some time a certain amount of chronic pancreatitis will likewise have developed. The condition is, nevertheless, of some importance in the diagnosis of chronic pancreatitis, particularly if fluid fat in large amounts is observed to separate from the stools.

Besides steatorrhea, the appearance of large numbers of undigested muscle fibers (azotorrhea) is frequently observed in chronic pancreatic disease. This is particularly suggestive if there is no associated diarrhea, and if the muscle fibers disappear upon the administration of pancreatin. According to several observers (Wohlgemuth, Einrequez, Ambard and Binet a. o) the quantity of fecal diastase is quite constantly diminished or absent.

The Urine.—After a most detailed investigation of the claims set forth by Cammidge regarding the diagnostic value of the reaction which bears his name the consensus of opinion seems to be that it is of little, if any value and should not be relied upon in the diagnosis of pancreatic disease. It may at times be met with in perfectly healthy individuals and occurs not only in disease in which the

pancreas is not involved, but may also be absent in cases where the pancreas is extensively diseased.

The conjugate sulphates are usually much diminished in pancreatic disease; so that their ratio to the mineral sulphates is materially increased (in 1 case of pancreatic calculosis 1 to 39.3 as contrasted with the normal 1 to 10). Le Nobel in one instance found no conjugate sulphates whatever. Pratt states that of 37 cases of pancreatic disease which had been studied by functional methods 6 showed spontaneous glucosuria and 4 the digestive form. Frequent examinations should be made as the transitory form is otherwise apt to be overlooked.

Glucosuria is observed only in advanced cirrhosis of the pancreas. Cammidge states that in the 90 cases of proved pancreatic disease investigated, sugar was only found in 5, all of which were examples of advanced chronic inflammation.

Acetone and diacetic acid also are not found in the absence of diabetes.

Bile pigment is present in a large percentage of cases, which is not surprising, considering the frequency of obstruction of the common duct as a causative agent of chronic pancreatitis. Albuminuria and cylindruria when present are referable to the accompanying jaundice. (See Cholelithiasis.)

The amount of diastase is increased in the urine during acute exacerbations of the disease.

PANCREATITIS (SUPPURATIVA)

The laboratory findings in suppurative lesions of the pancreas are essentially the same as in the hemorrhagic form. Abscess formation involving the head of the organ by compressing the common duct may give rise to a clinical picture which resembles that of cancer of the pancreas or of the common duct.

PARAGONIMIASIS

(Parasitic hemoptysis; pulmonary distomatosis; endemic hemoptysis)

In the pulmonary form of the disease the eggs of the corresponding parasite are found in the sputum, which is generally blood-tinged, and may also contain Charcot-Leyden crystals. In the abdominal type the ova may be found in the feces.

No adequate records exist regarding the condition of the blood or urine.

PARESIS

Essential Factors.—Progressive chlorotic anemia; hyperleukocytosis of the neutrophilic type with normal or increased eosinophile values

in the later stages; positive Wassermann reaction with blood and cerebrospinal fluid; cerebrospinal lymphocytosis; positive Noguchi (butyric acid) reaction with the cerebrospinal fluid.

The Blood.—*The Red Cells and Hemoglobin.*—During the earliest stages of the disease there is frequently no anemia whatever, but at the time when the patient first enters the hospital there is generally a moderate loss of hemoglobin, while the red count remains practically normal. As the disease progresses this chlorotic type of anemia increases and in the terminal stage of the disease the red count also may be subnormal. MacPhail found 67 per cent. of hemoglobin as average value on admission, and 52 per cent. toward the end, while the red count had dropped to from 3,000,000 to 4,000,000. Similar findings have been reported by others. Owing to blood concentration, no doubt, the laboratory findings do not always coincide with the patient's apparent anemia.

The Leukocytes.—The leukocytes are not increased during the early stages of the disease; later on, however, hyperleukocytosis commonly develops and reaches its highest point shortly before death. The majority of writers state that the increase is of the neutrophilic type, but, contrary to what we see in the septic infections, the eosinophiles are not diminished. They may, indeed, be increased (according to Roncoroni in some cases to 20 to 25 per cent.); Kippel and Lefas report an increase of the mast cells—up to 5 per cent., and maintain that later in the disease the neutrophilic hyperleukocytosis is replaced by a usually, relative lymphocytosis.

Serology.—In all suspected cases the Wassermann reaction should be tried, as this furnishes positive findings in virtually every instance, the complement fixation being almost invariably complete.

A positive Abderhalden reaction has been obtained in a large percentage of cases with brain as antigen. A reaction with liver and testicular tissue also seems to be quite common.

The Urine.—The urine shows no special abnormalities which can in any sense be regarded as characteristic. The mineral constituents and the urea output are essentially influenced by the patient's appetite and the quantity of food that is taken. According to H. Strauss and Arndt, digestive glucosuria (following the administration of 100 grams of glucose) is not uncommon in general paresis, but unfortunately, the number of cases which they examined was rather small, and J. Strauss, on the other hand, obtained negative results in all of 10 cases.

A transient albuminuria has also been described in general paresis, but we know nothing of its frequency, or any other details.

The Cerebrospinal Fluid.—Cytological examination quite constantly reveals the existence of a lymphocytosis, which may be an important factor in the differential diagnosis between this condition, on the one hand, and a simple neurosis or malignant disease on the other.

The Wassermann reaction shows complement fixation. Noguchi's butyric acid reaction and the Ross-Jones test likewise furnish a positive result.

PELLAGRA

The Blood.—A certain degree of chlorotic anemia is quite commonly observed in well established cases, but is not an essential factor in the clinical picture of the disease.

Hyperleukocytosis owing to an increased production of lymphocytes seems to be of frequent occurrence, but rarely exceeds 15,000 cells. Hillman's count vary between 6300 and 18,000. But even in these cases in which the total count is not above normal the percentage of lymphocytes is frequently increased. Hillman's maximal value (for the small lymphocytes) was 50 per cent. A polynucleosis is only exceptionally seen. In a case of this order which I had occasion to examine shortly before death the total count was quite low (2000 estimated), while the polynuclears numbered 85 per cent. The eosinophiles in Hillman's series varied between 0.60 and 9.0 per cent., with 2.94 as average. In 6 of the 37 cases the number was above the maximal normal, and it is noted that in some of these no parasites were found in the stools, while in others no examination was made in this direction.

The Wassermann reaction was negative in 2 cases which I had occasion to examine. This is in opposition to Bass, who speaks of a positive reaction in some of his cases which he was inclined to refer to the pellagra *per se*.

The Stomach Contents.—In 8 out of 14 cases Myers and Fine found anacidity which was generally associated with absence of pepsin or with the presence of minute quantities only.

The Urine.—In 45 per cent. of their cases Myers and Fine found hyaline casts. The indican was markedly increased, and the conjugate sulphates, especially high in those cases in which anacidity existed.

PERIODICAL PARALYSIS

The blood shows no abnormalities, barring a high degree of alkalinity, both during the attacks and in the intervals.

PERNICIOUS ANEMIA (BIERMER TYPE)

Essential Factors.—Oligocythemia with increased color index; macrocytosis with tendency to oval form; poikilocytosis; presence of erythroblasts with predominance of megaloblasts over normoblasts; basophilic granular degeneration; polychromasia; leukopenia; lymphocytosis; diminution of the plaques.

The Blood.—The blood picture of pernicious anemia is usually quite characteristic during the active stages of the disease; if, however, the patient is seen for the first time during a period of improvement or in the interval between attacks, the diagnosis may be very difficult, if not impossible.

The Red Corpuscles and Hemoglobin.—One of the most characteristic features is the lack of relation which usually exists between the decrease in the number of the red cells and the loss of hemoglobin. The former are always much diminished, when the disease is active, while the hemoglobin is generally affected to a less degree. The color index is accordingly increased. This will usually be found at the time when the patient for the first time seeks the advice of the physician, viz., relatively early in the course of the disease, while later, especially in the course of improvement in the patient's condition and in relapses, lower values may be found. Exceptions to this rule are, however, not uncommon. In Cabot's series of 139 cases a color index higher than 1 was apparently present in 93, or 71 per cent., while one lower than 1 was found in 41, or 29 per cent., of the cases; his average was 1.04. Exceptionally it may rise to nearly 2. The red count on first examination is usually well below 2,000,000; in many instances the initial count is but little higher than 1,000,000. Subsequently rapid and extensive changes may occur. It is remarkable to see a patient one day with his red cells near 1,500,000, and only a few months later about the 4,000,000 mark or higher. On the other hand, the count may fall as rapidly, and toward the fatal end extraordinarily low counts have been recorded. Values between 500,000 and 1,000,000 are not at all uncommon. In one case reported by Quincke a count of 143,000 was observed; seventy-four days later the patient had 1,234,000 per c.mm. Osler reports a case where shortly before death the count fell below 100,000; this is the lowest count that has been recorded. In a series collected by Strauss and Rohnstein, 1,240,000 was the average at the time when the patient first came under observation, and in Cabot's series the average number was almost identical—1,200,000.

As I have already emphasized, the hemoglobin values are usually higher than the red counts. In the series of Strauss and Rohnstein the average value was 25 per cent., and in 9 cases of the 23 it was lower than 20 per cent. I have repeatedly noted 15 per cent., and A. Meyer reports a bothriocephalus case with only 10 per cent.

Morphological examination shows a marked tendency to anisocytosis. The general blood picture is distinctly macrocytic; during the active period of the disease, in fact, the macrocytes may represent 70 per cent. of all red cells. The condition, however, is not constant; during periods of improvement the macrocytes diminish markedly in number, and in the remissions they may disappear. Corresponding to the general macrocytosis the *volume index* is increased. In

29 cases examined by Capps it ranged between 1.05 and 2 during the active stage of the disease; it fell during periods of improvement and rose in periods of decline. Similar observations have been recorded by Wroth.

Poikilocytosis is usually also quite marked; but it is really the anisocytosis which is characteristic, and together with this a remarkable tendency to *oval form*. The macrocytosis may, in a general way, be viewed as the morphological equivalent of the increased color index, but it is noteworthy that practically *all* cells are well colored; the pessary forms of the severe secondary anemias with pale centres do not belong to the blood picture of pernicious anemia; they are rarely seen.

In the fresh specimen *money-roll formation* is usually much delayed or may be absent; this, however, is not characteristic.

In stained specimens *granular degeneration* of the red cells is very commonly seen. I know of no disease in which it is so constant or so extensive. It may be demonstrable in the intervals and at a time, even when the blood picture is otherwise nearly normal. Such cells may be encountered whether nucleated red cells are simultaneously present or not. I found them most numerous in a patient in whom blood crises occurred; nucleated as well as non-nucleated cells were equally extensively involved. My impression has been that the occurrence of granular degeneration in pernicious anemia is intimately connected with blood regeneration, and that it is absent when this becomes arrested. *Sub finem vitæ* and in the so-called aplastic cases of the disease it is usually lacking. Occasionally Cabot's *ring bodies* may be seen; their search, however, may be tedious. *Polychromasia* of the red cells is common and in the megaloblasts practically constant.

Excepting the aplastic cases and *sub finem vitæ, nucleated red cells* are almost always found while the disease is active. Their number, however, is extremely variable. In many cases it is necessary to run over many slides, taken on different days, before a single nucleated cell is found, while in others a dozen or more may be encountered within a narrow compass, and during blood crises they may be very numerous. A careful examination will show that megaloblasts outnumber the normoblasts in nearly all cases. In Cabot's series of 139 cases they predominated in 109 at the time of the first examination, and in 27 of the remaining cases at a later period. In Da Costa's series of 81 cases the same was found in 76. While the *predominance of megaloblasts* is unquestionably one of the most important factors in the blood picture of pernicious anemia, their occurrence is not pathognomonic of this disease, as was once supposed. The modern tendency is to regard their appearance in the blood merely as evidence of an anemia of unusual severity, viz., as a degenerative-regenerative phenomenon. While many of the megaloblasts

are young cells, of the type of the large lymphocyte, the old megaloblasts, of the type of the large mononuclear leukocytes, are really the most characteristic. The normoblasts frequently are seen undergoing karyolysis, as well as karyorrhexis. Mitotic red cells are not uncommon.

During the periods of amelioration and in the intervals between attacks the nucleated red cells may virtually disappear from the circulating blood, but at times it may be possible even then to find an occasional megaloblast and thus clinch a doubtful diagnosis.

The Leukocytes.—In contradistinction to many of the secondary types of anemia, the leukocytes in pernicious anemia are usually diminished in fully 65 per cent. of all cases (2000 to 4000). As Da Costa remarks, the number of the leukocytes run a course roughly parallel to the number of the red cells, increasing to normal or maximal normal values during periods of improvement, and falling again as the disease progresses. Complications may cause an increase in the number, and the same may be seen toward the fatal end; higher values than 12,000 or 15,000 are, however, exceptional even then. In some instances the leukopenia is extreme. Strauss and Rohnstein cite two cases with 400 and 328 cells respectively, and Da Costa mentions a case in which no leukocytes whatever could be found. The decrease in the total number of the cells is referable to a diminished formation of granular leukocytes in the bone marrow, and we accordingly find these diminished, while the mononuclear non-granular forms are relatively increased. This increase usually affects the lymphocytes exclusively, but occasionally the splenocytes also are increased. Combining the two, Cabot's average of 52 cases was 45.4 per cent. With the occurrence of improvement the number may diminish, while toward the fatal end it may rise still farther, viz., to 70, 80, or 90 per cent. Williamson and Martin note a count with 99 per cent. Neutrophilic myelocytes are usually present in small numbers, but occasionally 6, 8, and even 10 per cent. have been counted. More common than the myelocytosis is the frequently very marked polymorphism of the neutrophilic leukocytes, a factor which has some weight in diagnosis, as in the secondary anemias a normal number of lobes seems to be the rule (Briggs). The eosinophiles are frequently diminished, while in other cases the marrow insufficiency affects only the neutrophiles, leaving normal eosinophile values; increased values are occasionally seen and may be due to complicating conditions.

The Plaques.—The plaques are usually diminished in pernicious anemia. In two cases von Embden found only 64,000 and 32,000 respectively. At times they are apparently absent, but in some cases increased numbers have been observed. According to Pappenheim the usual diminution of [the plaques in pernicious anemia is referable to over-rapid] maturation of the red cells.] As a consequence

the nuclei of the erythroblasts either do not become pyknotic and undergo subsequent chromatolysis with consequent formation of nucleoids, but are destroyed already at an early stage by karyorrhexis; or if they do become pyknotic they are expelled from the cells plasmolytically in the anisotonic, anemic blood serum. A nucleoid thus does not remain in either case which could later escape as a plaque.

Serum.—The blood serum in pernicious anemia usually contains bilirubin and frequently also urobilin, both of which can be readily demonstrated.

The Feces.—The examination of the feces should never be neglected, as two intestinal parasites have been demonstrated to be possible causative factors of pernicious anemia, viz., the hookworm (Uncinaria duodenalis) and the broad tapeworm (Bothriocephalus latus.) Their search is usually a simple matter. (See Technique and section on Helminthiasis).

Of chemical constituents urobilinogen is the most interesting as it constitutes an index of the extent of hemolysis that is going on.

The Urine.—The urine shows no changes which are characteristic. Indican is usually demonstrable in increased amount, and Hunter reports that he has found putrescin and cadaverin in the urine. Urobilin is present during the active periods of the disease, while extensive destruction of red cells is taking place. Albuminuria is exceptional. Leucin, tyrosin, acetone, and diacetic acid have been found in isolated cases, while sugar is absent. The diazo reaction is negative.

PHARYNGOMYCOSIS LEPTOTHRICIA

In the pyoid masses derived from the crypts of the tonsils in cases of follicular tonsillitis, and also in persons who have had frequent attacks of tonsillitis, large numbers of leukocytes of all sizes are seen, besides epithelial cells and long, segmented fungi, the Leptothrix buccalis, which are colored bluish red by a solution of iodopotassic iodide. At times, patches composed of these fungi extend over a considerable area of the tonsils, so that it may be doubtful whether or not the disease is a beginning diphtheria.

More extensive invasions have been described by Dubler, who noted a Leptothrix mycosis involving the pharynx, œsophagus and larynx; and by Baginsky, in the case of the pharynx, trachea, and nose.

PNEUMONIA (PNEUMOCOCCUS)

Essential Factors.—Hyperleukocytosis; septic factor; increased fibrin formation; pneumococcemia; rust-colored sputum, with predominance of the pneumococcus; low chloride content of the urine.

The Blood.—*The Red Cells.*—As in many other febrile diseases, the number of the red cells is usually not found reduced during the active stage of the disease, although actual corpuscular destruction may be going on at the time. In other words, there is a relative polycythemia. The anemia, however, becomes apparent as defervescence occurs, and may continue well into convalescence. Usually it is of moderate grade, but occasionally it is quite severe. A loss of 500,000 to 1,000,000 red cells per c.mm. may be regarded as moderate. Normoblasts are occasionally seen in small numbers.

Hemoglobin.—The type of anemia is the chlorotic form, viz., the loss of hemoglobin exceeds that of the red cells. Following defervescence it is commonly reduced to 70 per cent.; but it may be much lower.

The Leukocytes.—Hyperleukocytosis is one of the most constant symptoms of pneumonia, and is seen in nearly all cases during the active stage of the disease. It may, however, be absent in very mild infections and exceptionally in those severe cases in which the patient is apparently overwhelmed by the intensity of the toxemia at the very outset of the disease, and dies without any evidence of active resistance. The increase of the leukocytes occurs early in the disease. I have repeatedly noted it on the first day. Ewing mentions an instance in which 25,000 cells were counted within four hours of the initial chill. As a rule, it persists with moderate fluctuations throughout the active stage of the disease, until a few hours before or up to the time of the crisis; it then falls quite abruptly, but not as rapidly as the temperature. Pseudocrises, on the other hand, are not usually associated with a drop of the leukocytes, but exceptions occur. If defervescence takes place by lysis, the decline is more gradual and the temperature reaches the normal before the leukocytes. In cases of delayed resolution the hyperleukocytosis persists. The occurrence of complications, in the course of the disease, or the involvement of new areas of lung tissue, is generally marked by corresponding rises in the count.

The actual number of the leukocytes varies very much; 17,500 to 22,500 may be regarded as average values. The number may, however, be much larger. In Cabot's series of 842 cases 14 are recorded in which it exceeded 50,000, and in one more than 100,000 were counted. In children the figures are, *cæteris paribus*, higher than in adults.

Generally speaking, the extent of the hyperleukocytosis is proportionate to the amount of lung tissue involved, but there are numerous exceptions. Ewing states that in 63 cases in which one lobe only was affected the average count was 20,000; in 24 cases with two lobes involved, 22,700; in 12 with three lobes consolidated, it was 25,000; and in 1 with four lobes, 27,000. In one instance with bronchial breathing over the entire back the count was 32,000. On the other hand, I have found a leukocytosis of 30,000 and more in several

cases at a time when no consolidation could as yet be demonstrated (so-called central pneumonia).

From a prognostic standpoint, high leukocyte values may be regarded as evidence not so much of an especially intense infection as of active resistance on the part of the individual. Hypoleukocytosis, when following a hyperleukocytosis, is a correspondingly unfavorable symptom, as it is the expression of an exhaustion of the defensive power of the body.

The leukocytosis in pneumonia is of the polynuclear neutrophilic type, with absence or marked diminution of the eosinophiles. This association I have for many years spoken of as the septic factor, and I would emphasize that there is no bacterial infection in which it is more constant and more pronounced than in pneumococcus pneumonia. Even in the mildest cases, in which the total number of the leukocytes may be little, if at all, above the normal, the septic factor is well pronounced. In cases of average severity the neutrophiles are rarely below 80 per cent.; in most cases they are nearer 90. Early in the disease I have frequently found them above this figure. These high values are, indeed, so common in pneumonia that in their presence I invariably suspect the existence of the disease, even though no sign of consolidation can be made out.

The Arneth count shows a marked predominance of the polymorphonuclear forms, while the polynuclear elements proper are much diminished. Metamyelocytes are frequently seen during the acme of the disease. They are especially common in children, but rarely exceed 3 per cent. They may be more numerous about the time of the crisis. In one instance I found 19.6 per cent. on the second day after crisis.

With the establishment of convalescence the neutrophiles usually drop below the normal, while a proportionate increase of the small mononuclears, which were previously much diminished, occurs.

The eosinophiles, which during the active stage of the disease almost disappear from the blood, sometimes return to normal minimal values even before the crisis is reached, and during convalescence they commonly rise above the normal for a few days.

It has been reported that in children lymphocytosis takes the place of the neutrophilic polynucleosis. I must confess that I doubt such an occurrence in actual pneumococcus infections. I believe that future investigations will show that the pneumococcus is not the offending organism where a lymphocytosis exists.

Iodophilia is the rule in pneumonia. After crisis with good resolution it disappears in twenty-four to forty-eight hours.

The Plaques.—The plaques are diminished during the acme of the disease; but about the time of the crisis and immediately following they are usually much increased. Smear preparations may then be full of them.

Fibrin.—The tendency to fibrin formation is so commonly increased in pneumonia as to constitute one of the diagnostic factors of the disease. It is absent, however, in those cases in which hyperleukocytosis is wanting. It often continues well into convalescence.

Bacteriemia.—While it has been claimed that with suitable methods the pneumococcus can be demonstrated in the blood of all pneumonia patients at some time in the course of the disease, it is no doubt the experience of most laboratory workers that negative results are after all very common. And while the appearance of the organism in the blood does not necessarily involve a fatal ending, positive findings are unquestionably more common in fatal cases than in those ending in recovery.

Positive results may be reached as early as twelve hours after the initial chill. After the crisis the findings are usually negative, but the organism has been found as late as forty-eight hours after the decline in the temperature. While culture should be resorted to in doubtful cases, it is interesting to note that Rosenow found pneumococci in blood *smears* in 47 cases out of 80 examined; no phagocytosis, however, was demonstrated.

The number of organisms which may be found is variable. While frequently small, extraordinary numbers are at times encountered. This is most apt to be the case shortly before death when the plate may show thousands of colonies. Dochez reports that his highest count was 65,000 colonies per c.c. of blood, obtained at the time of death on the eighth day of the disease.

Cryoscopic Examination.—The freezing point of the blood is owered. A return to the normal does not occur until several days after the crisis.

Alkalinity.—According to some observers the alkalinity of the blood is reduced in pneumonia; others report normal values.

The Sputum.—With the exception of young children and the aged, there is expectoration of sputum in most cases of pneumonia, but not in all. I have seen an instance in which the patient passed through the entire course of the disease, lasting nearly three weeks, with no expectoration at any time. In the Hopkins series little or no sputum was obtained in 16 per cent. of the cases. When present it may at first be mucoid, but sooner or later it becomes bloody to a greater or less degree, and in fully 30 per cent. of the cases it is distinctly rusty in appearance. It is then so characteristic as to constitute one of the diagnostic factors of the disease; it is quite homogeneous, nearly transparent, and so tenacious that the cup can frequently be inverted without spilling a drop. With more extensive involvement of the bronchi the sputum becomes mucopurulent, less tenacious, and more abundant, and at the time of the crisis the rusty color disappears altogether. With the involvement of new areas of

lung tissue it is again more or less bloody. The appearance of the rust color, of orange-yellow, lemon-yellow, and grass-green shades, is referable to the formation of unknown oxidation products of hemoglobin; the green and yellow tones may, however, be associated with jaundice. So-called prune-juice sputum has long been regarded as an evil omen. It is seen, as a matter of fact, in especially severe cases and in those in which active resistance is much impaired; it may, however, also be found in ordinary cases with beginning resolution. When abscess or gangrene of the lung develops, the sputum becomes fluid; it assumes a coffee color, then that of prune juice, and ultimately it is chocolate brown. (See Gangrene and Abscess of the Lung.)

Fibrinous coagula, according to Osler, are constant constituents of pneumonic sputa, but are usually overlooked. Curschmann's spirals also may be present.

The amount of sputum in the twenty-four hours varies between 150 and 300 c.c., increasing in proportion to the degree of bronchial involvement.

Chemical Examination.—Chemical examination shows a large amount of soluble albumin, an increase of the mineral solids (about 26 per cent.) as compared with other sputa (viz., about 18 per cent.), in which the sulphates and potassium salts play a prominent role, while alkaline phosphates are absent.

Microscopically, there are numerous epithelial cells, leukocytes, and red corpuscles, many of them in various stages of degeneration, and most important of all large numbers of pneumococci. This organism is practically a normal inhabitant of the mouth and the upper air passages, and is accordingly always present in the expectoration. Its demonstration *per se* is hence of little interest. In pneumonia, however, it is present in such large numbers, and often in almost pure culture, as to constitute a proper diagnostic factor.

The Urine.—In its physical aspects the urine of pneumonia presents those characteristics which are referable to the febrile state *per se*. During the active stage of the disease it is thus commonly of a moderate or dark amber color, clear when voided, of a more or less aromatic odor, and a very decidedly acid reaction.

On standing, heavy sediments of urates are prone to form. The specific gravity is high—1024 to 1026—throughout the course of the disease, and does not fall materially until defervescence. This is sometimes preceded by several hours by a sudden *polyuria* with low specific gravity. Pseudocrises, however, may be associated or preceded with a similar increase in the amount of urine and a correspondingly low specific gravity. In the severest cases the amount of urine is materially diminished and does not exceed 850 c.c. In average cases the patients pass in the neighborhood of 1000 c.c. The *solids* in fatal cases are usually somewhat less than 50 grams. In the others

the amount is somewhat higher—60 grams or thereabouts; it is less during convalescence and defervescence. The amount of *urea* is high; 32 to 40 grams may be regarded as average values during the active stage of the disease, while in convalescence the figures are still further increased. In this connection the researches of H. W. Cook are of special interest. In his study of the nitrogen excretion in pneumonia and its relation to resolution he arrived at the following conclusions: (1) In cases of pneumonia a surplus amount of nitrogen must be excreted during the days of resolution that will correspond at the least to the original quantity of the exudate poured into the involved lung. In most cases there is more, the rest representing, in great part, a continuation of the formation and an absorption of inflammatory exudation plus other tissue destruction. (2) In cases of marked delay in resolution the continued high nitrogen output indicates a continuation of the local inflammatory process, so that in those cases of several months' persistence we might speak of a chronic pneumonia. (3) In cases of rapid resolution the leukocytosis curve follows the curve of nitrogen excretion with a very striking parallelism, and would seem to point to a causal relation between leukocytes and resolution.

During the febrile period the organic acidity of the urine is markedly increased; this may continue after the temperature has returned to normal, while in other cases the phosphatic acidity is then greater than the "total acidity."

The amount of *uric acid* is very commonly increased, and may reach 2 grams in the twenty-four hours. The inorganic solids average 5.8 grams during the active stage and 10.8 grams during convalescence. The *chlorides* are notably diminished, and may at times be absent. Robin gives 0.95 gram as average value in fatal cases, and 1.2 in those which recovered. This decrease is so constant as to constitute one of the diagnostic factors of the disease. During convalescence there is again an increase, after which there is a gradual return to normal. The *phosphates*, as a whole, are diminished. Vogel gives 1.417 grams as average. The sulphates are apparently increased, but have not been studied in detail. The amount of *indican* is increased in fully 50 per cent. of all cases. It may be normal, however, throughout the disease, even in the fatal cases. *Urohematin* and *uroërythrin* are constantly increased and often present in notable amounts. *Albuminuria* is very frequent in pneumonia, and at times the amount is considerable. According to Jaccoud, it is of more frequent occurrence than in any other disease of the respiratory organs. In a series of 799 cases reported from the Boston City Hospital it was found in 624; that is, in 78 per cent. of the cases. It was noted that the death rate bore a direct relation to the amount of albumin. Hyaline tube casts are common. The leukocytes are usually somewhat more numerous than normal, and at times isolated

red cells are found (in severe cases). Sugar is absent; a digestive glucosuria, however, can at times be produced. The *diazo* reaction is occasionally seen with the ordinary method of dilution, viz., 1 to 40, while according to Greene it is rarely found if 1 part of the nitrite solution is added to 100 parts of the sulphanilic acid solution. With this modification he obtained only one positive result in 11 cases. In my own experience the color reaction is rarely as intense in pneumonia as the typical typhoid reaction, and it is noteworthy that it develops earlier than in typhoid fever. The *benzaldehyde reaction* is likewise occasionally seen, as is also the *egg-yellow reaction*, but neither can be viewed as of diagnostic importance. In pneumonia, uncomplicated by hepatic disease, the *urobilin* curve is only slightly above the normal at the beginning of the disease, high during the crisis, with a rapid return of the normal, coincident with the termination of resolution. In the event of a complicating hepatitis at the beginning of the disease or after resolution the urobilin curve goes up. This observation early in the disease is of considerable importance from the standpoint of prognosis.

POLIOMYELITIS (ACUTE EPIDEMIC)

Essential Factors. — Hyperleukocystosis of the polynuclear type. Increased cell count in the cerebrospinal fluid with a tendency to the lymphocytic type.

The Blood.—According to Peabody, Draper and Dochez a marked leukocytosis is, generally speaking, a constant feature of the disease and persists for a remarkable length of time. Their total counts varied between 8800 and 35,000 in preparalytic and abortive cases, and between 10,700 and 24,000 during the first eight weeks of the disease, the highest counts occurring during the first week, from which time off the number gradually diminishes, but continues well above the normal for a long time. In one instance a count of 16,000 was noted during the twelfth week.

The increase in the total number of the leukocytes is in part no doubt due to an increase (of from 10 to 15 per cent.) in the number of the neutrophiles, when contrasted with the normal values of children of corresponding ages, but in some cases the total increase seems to be due to accidental factors rather than to an actual increased production of any one type.

The behavior of the eosinophiles is quite inconstant—sometimes they are found greatly diminished, at others they are normal and in still other cases increased.

The Cerebrospinal Fluid.—The cell count is increased in most cases during the first days, after the onset of the symptoms, and preceding that of the paralysis, after which there is a steady decline, normal values being the rule by the end of the second week. The actual

number may vary considerably. During the first week it is above 50 in 50 per cent. of the cases, while the majority of the remainder give counts varying between 12 and 50. During the second week already values above 50 are obtained in but 17 per cent., while in approximately one-half of the cases the values are lower than 50, but higher than 12. In the remainder normal figures are obtained.

More important than these data is the observation that very high counts are apt to be found during the prodromal period. Peabody, Draper and Dochez thus mention three cases with counts of 990, 627 and 1221 respectively. While the predominating cell at the outset may be the polynuclear neutrophile, it is more common to find a lymphocytosis, and even in those cases in which a polynucleosis occurs at first this is soon replaced by a lymphocytosis.

Generally speaking, an increased cell count is associated with a low or normal globulin content and *vice versa*.

All fluids examined were found to reduce Fehling's solution.

During the acute stages the fluids of 40 to 50 per cent. of the cases show a colloidal gold reaction of the luetic and meningitic zone types (Kolmer and collaborators).

PREGNANCY AND THE PUERPERAL STATE

Essential Factors.—Tendency to hyperleukocytosis affecting all types of leukocytes; presence of protective ferments in the blood; lactosuria; tendency to digestive glucosuria; acetonuria and increased ammonia content of the urine in the toxemic type of the vomiting of pregnancy; lochial discharge.

The Blood.—*The Red Cells and Hemoglobin.*—Pregnancy *per se* does not affect either the number of the red cells or the amount of hemoglobin. When anemia exists, this is due to complicating factors or to unhygienic conditions. After birth there is a variable degree of anemia, which persists for a week or two and then disappears. The former view that pregnancy may be a causative factor of pernicious anemia has been abandoned. When severe anemia develops after pregnancy it is always of the secondary type and referable to complicating conditions. Extreme and rapidly progressive anemia is noted in streptococcus infections. There are few conditions, in fact, in which the destruction of the red cells proceeds so rapidly as in puerperal infections of this order. (See Septicemia.)

The Leukocytes.—An increase in the number of the leukocytes, particularly marked during the last five months of pregnancy, appears to occur quite constantly in primiparæ, while in multiparæ exceptions are common. In an analysis of 55 cases, Hubbard and White found hyperleukocytosis in 44 (80 per cent.), which was most marked and constant in young primiparæ. Rieder noted an increase in 20 out of 31, all the negative cases being multiparæ. In a series of 17 multiparæ an increased number was noted in only 7. In Rieder's series the number varied between 10,000 and 16,000 (13,000 average).

This represents the usual increase. At times the numbers may be much larger. Cabot thus reports three cases with a leukocytosis of 25,000 to 37,000. Lobenstein gives the following results from an analysis of 50 cases in the ninth month:

Average count, 10,600; highest count, 18,000; lowest count, 5400; average in primiparæ, 9346; average in multiparæ, 11,854; absence of leukocytes in 7 cases.

During actual labor there is an increase of the leukocytes over and above the numbers previously observed in pregnancy; 30,000 cells may be noted.

Lobenstine's figures, in his series of 50 normal cases on the third day of the puerperal period, are the following:

Average count, 12,400; highest count, 20,400; lowest count, 5600; average in primiparæ, 13,200; average in multiparæ, 11,600; no leukocytosis in 8 cases.

The highest numbers are met with in severe and protracted cases, especially after rupture of the waters. This form of hyperleukocytosis subsides after the expulsion of the child, and at the end of the first half of the second week, normal values are again reached, though the gradual decline may be interrupted by a temporary increase now and then, referable to various minor disturbances during the puerperal state. In many cases normal values are reached much earlier, and by the third day, as a rule, the number is as low as it was before labor. The increase in the number of the leukocytes, both in pregnancy and during the puerperal state, seems to be a general increase, affecting both the mononuclear and the polynuclear elements.

When *septic complications* supervene, hyperleukocytosis with the septic factor are of constant occurrence.

In *eclampsia* there is usually marked hyperleukocytosis, the degree, *cæteris paribus*, depending fairly closely upon the apparent toxicity of the case (16,000 to 20,000 in mild cases). With a good resistance, the increase is especially marked (46,000 to 54,000). A sudden increase generally indicates an aggravation of the condition in an individual of good resistance (as high as 100,000). A low count in a highly toxic patient is of bad omen (19,000 dropping to 13,800 by the second day following delivery). A leukocytosis originally high that falls rapidly in a badly toxic patient is likewise a danger signal (100,000 to 45,200 in one day; Lobenstine).

In the differential diagnosis between *ruptured tubal pregnancy* with associated internal hemorrhage and acute peritonitis, a high leukocyte count speaks in favor of the first condition. In a slowly developing peritonitis, on the other hand, hyperleukocytosis may also be observed. With small hematoceles (referable to tubal pregnancy) the leukocytes may be normal.

Coagulation.—The coagulation time in healthy pregnant women is virtually the same as in non-pregnant individuals, and there is no material difference in eclamptic, as compared with non-eclamptic, cases.

Serology.—Abderhalden Reaction.—Through the researches of Abderhalden it has been established that as a result of the pregnant state certain *ferments* appear in the blood which are characterized by their specific proteolytic action upon placental proteins. They can be demonstrated in their effect upon placental tissue either polarimetrically or by the so-called ninhydrin reaction (see Abderhalden's methods in section I). As a result of the study of some 2000 cases by various observers we may conclude that the reaction is specific, providing that the technique of Abderhalden is followed in every detail. It usually appears during the first month and persists until the end of the third week following delivery. It is also present in cases of *extra-uterine pregnancy.*

In *eclampsia* Abderhalden found that the serum also gave a marked reaction with liver tissue and exceptionally with thyroid.

The Urine.—In the majority of cases of strictly normal pregnancy the urine shows no material deviation from the normal, barring the development of a variable degree of *lactosuria* and a common tendency to digestive glucosuria. Lactose may, indeed, be viewed as a normal constituent during the last weeks of pregnancy and the first weeks following childbirth. The antepartum lactosuria usually amounts to about 1 gram per liter, but may reach 2 grams, or even higher figures, though this is rare. The maximum amount is met with between the third and fifth days after labor, the quantity varying between 1 and 8 grams per liter. After lactation is once well established, lactose is not usually found in the urine, but it may occur if for any reason milk stasis develops.

Occasionally the lactosuria is accompanied by a mild grade of *glucosuria.* Digestive glucosuria, on the other hand, is a fairly constant symptom and of some diagnostic importance. Its extent is variable. While Lanz has recorded a case in which 29.6 grams of glucose were eliminated after the ingestion of 100 grams, such figures are uncommon, and, as a general rule, less than 3 grams are recovered from the urine. After delivery, the power of assimilation for glucose no longer appears to be subnormal. *Albuminuria* is observed in about 50 per cent. of all cases of pregnancy, and is of equal frequency in both primiparæ and multiparæ. During labor the condition is even more common. Hyaline cylindruria usually accompanies the condition. The amount of albumin is small and rapidly disappears after delivery in those cases in which no albumin was noted before, or in which it appears relatively late, while it frequently persists for a long time in those cases where the albuminuria developed early and was particularly marked. *Albumosuria* of slight grade is said to occur in about 25 per cent. of pregnant women, while during the second and third day of the puerperal period it is more frequent and the amount larger (Fischel). A small amount of *acetone* is found normally during the first two days of the puerperal period, but usually

disappears by the third day. Larger quantities may be met with in connection with the *pernicious* type of vomiting of pregnancy and in eclampsia (Baginski). In the toxemic form, which is characterized by marked degenerative changes in the liver, Williams found a large increase in the *ammonia* content of the urine (up to 20 to 45 per cent. of the total nitrogen), while this does not occur in the other forms of vomiting of pregnancy and in eclampsia. Other observers have corroborated his findings, and it has now been established that recovery may follow a timely diagnosis of the condition and emptying of the uterus, when the ammonia frequently drops at once. According to Williams, 16 per cent. may be regarded as the danger line. Prompt action at as early a period as possible is indicated, as otherwise, a fatal result may not be avoided

A slight rise of the ammonia occurs also during normal pregnancy and reaches its maximum during labor.

The urea is usually below the average normal in *eclampsia*, varying between 46.1 and 74.5 per cent. of the total nitrogen (van Slyke). It is to be noted, moreover, that the only eclamptic urines which showed more than 70 per cent. of urea nitrogen were those obtained one or two days postpartum. The ammonia is often higher than in the average normal individual, but the increase is not striking.

The Vaginal Discharge.—The *reaction* of the vaginal discharge is probably always acid during pregnancy. In 500 cases which Krönig examined in this direction an alkaline reaction was never obtained.

The question whether or not *pathogenic organisms* may occur in the vagina of pregnant women *may* be answered in the affirmative, but, with the exception of the gonococcus, they are not often seen. Bergholm thus examined the discharge in 40 cases, and was unable to demonstrate organisms pathogenic for animals in a single case; there were no pyogenic staphylococci, no streptococci, and no colon bacilli. Even when artificially introduced they rapidly disappear. Krönig thus found that the Bacillus pyocyaneus disappears from the vagina of pregnant women in ten to thirty hours, and the streptococcus within six hours. Important, from a therapeutic stand, is the fact that the bacteria disappeared less rapidly when the vagina was irrigated with water or even with antiseptics.

At times a profuse *blennorrhea* is observed during pregnancy which may assume a virulent character; the secretion then readily becomes purulent. At times this catarrhal condition is complicated with a mycosis, when white or yellowish-white patches may be seen at the orifice; the latter may, indeed, be occluded by conglomerations which consist entirely of fungi.

The Lochia.—The lochia during the first day following parturition are red in color—the *lochia rubra*—and emit the characteristic sanguineous odor. At this time a microscopic examination will reveal an abundance of red corpuscles, some leukocytes, and a variable

number of epithelial cells, which are almost exclusively of vaginal origin. On the second and third days the number of red corpuscles diminishes, while the leukocytes increase in number. Still later, the diminution in the red cells and the increase in the white corpuscles become more marked, and the discharge at the same time assumes a grayish or white color, until about the tenth day the red corpuscles have almost entirely disappeared, while the leukocytes and epithelial cells are abundant. Finally, the secretion becomes thicker, mucoid, and milky white in color—the *lochia alba*—which condition may persist for three to four weeks in nursing women, and still longer in those who do not nurse, until finally the normal secretion is again established. Numerous bacteria are encountered in the lochia, and it is curious to note that among these, pus organisms are quite constantly present without giving rise to symptoms. When a portion of the placenta or membranes have been retained the lochia soon give off a fetid odor, and assume a dirty brownish color; the retention of blood clots alone may also produce this result. In such cases the lochia swarm with bacteria of all kinds.

Abortion.—In cases of abortion it is often possible to discover *chorion villi* in the expelled blood clots presenting the characteristic capillary network, and often manifesting signs of advanced fatty degeneration. Important, also, from a diagnostic point of view is the presence of *decidual cells*, which are characterized by their large size, their round, polygonal, or spindle-like form, and their characteristic nuclei and nucleoli.

When *septic infection* occurs, bacteriological examination of the lochial discharge will reveal the presence of the offending organism. Those mostly concerned are the streptococcus, the Staphylococcus aureus, and the colon bacillus.

PURPURA HÆMORRHAGICA

Essential Factors.—Irregular anemia; irregular hyperleukocytosis, with variable leukocytic formula; diminution in the number of the plaques; impaired coagulation.

The Blood.—*The Red Cells and Hemoglobin.*—In uncomplicated cases the number of red cells and the amount of hemoglobin are comparatively little reduced, and it is noteworthy how rapidly losses of blood are made up. Exceptionally, there is severe anemia. Ajello has described cases with a count of 2,500,000 to 3,000,000. Osler mentions one case with a count of only 1,800,000, and in Muir's patient the red cells dropped to 800,000, with 11 per cent. of hemoglobin. Whether this latter was not a case of aplastic pernicious anemia may be questioned. In markedly hemorrhagic cases normoblasts and polychromatophilic red cells may be encountered.

The Leukocytes.—The leukocytes are usually increased. Regarding the differential count there are no data. Barjou and Cade have described a case of acute infectious purpura in which the red cells fell to 2,027,000, and the leukocytes rose to 85,000, with 94 per cent. of polynuclears. In Muir's case the count was 7000, with 75 per cent. of mononuclear elements. In another case, described by Lipanski, there was a lymphocytosis amounting to 97 per cent. Here, also, there is some doubt about the nature of the case.

The Plaques.—The plaques are much diminished at the height of the disease.

In one case of the disease Ajello noted the occurrence of *methemoglobinemia.*

In cases which develop on the basis of acute infectious diseases the corresponding *organisms* may be found in the blood.

Coagulation.—Primary coagulation occurs as with normal blood; subsequent retraction of the clot and exudation of the serum, however, take place only to a limited extent. While normal serum added to fluids, such as hydrocele fluid, which are not spontaneously coagulable (in the proportion of 1 to 80) induce coagulation in four to six minutes, the serum of purpuric patients is either entirely devoid of this property, or possesses it only to a very slight degree. The addition of a trace of calcium chloride, however, causes such a serum to behave much like normal serum. Sicard, hence, suggests that in certain cases of purpura the fibrin ferment or its proenzyme is not present in sufficient quantity to cause more than a primary coagulation.

The Urine.—In many cases no abnormalities are noted, while in some hematuria of greater or less extent is a feature of the symptom complex.

PYELITIS AND PYELONEPHRITIS

(Nephrolithiasis; renal calculus)

Essential Factors.—Irregular anemia; neutrophilic hyperleukocytosis; pyuria; hematuria; bacteriuria.

The Blood.—When pyelitis and pyelonephritis develop secondarily, either as the result of an ascending infection, or as a complication of a general infection, the blood picture will depend essentially upon the nature of the underlying malady.

The independent effect of a pyelitis upon the blood picture is best studied in cases which result from mechanical causes, viz., in cases of calculous pyelitis. In these there is no deviation from the normal for a long time, excepting during the occurrence of attacks of renal colic, when hyperleukocytosis may develop. In four of the Hopkins series, mentioned by Emerson, the counts ranged between 12,000 and 18,000. How constant this factor is remains to be determined; thus far there is practically no literature upon the subject. In the

interval between attacks the count is normal, unless the disease has advanced to a point where extensive ulceration has occurred or a complicating suppurative nephritis (pyelonephritis) has developed. In such cases high leukocyte values are almost always met with (15,000 to 30,000). The general health is then usually more or less impaired and anemia, frequently of considerable severity, common. The leukocytosis is of the neutrophilic type, with diminution or absence of the eosinophiles, such as we see it in other septic infections. Whether or not the eosinophiles are also diminished in the early cases during an attack of colic, I have not been able to ascertain.

The Fibrin.—The fibrin is usually increased in calculous pyelitis, in contradistinction to cancerous cases.

The Bacteriology of the Blood.—The bacteriological findings in the blood are negative, excepting in those cases where the pyelitis has developed as a complication of a general bacterial infection, when the corresponding organisms may at times be met with, as has been detailed in the corresponding diseases. *Sub finem vitæ,* when a general septicemia has developed secondarily to an originally non-bacterial pyelitis, agonal invasion of the circulation at large may be observed, but seems to be exceptional.

The Urine.—In *acute cases* there is a diminution in the amount of urine, which may at times go to the point of complete anuria, even though the inflammatory process be limited to one side; in bilateral cases this may persist and lead to the development of uremia. Owing to the presence of pus, the urine is more or less turbid; usually it is also mucinous and frequently hemorrhagic. In calculous cases there may be abundant deposits of uric acid, or calcium oxalate, or both. Fibrinous casts from the pelvis or ureter or bits of necrotic mucous membrane are more rarely seen (fibrinous or diphtheritic pyelitis). Chemical examination shows a fair amount of albumin (up to 0.3 per cent.), which may be further increased if much blood is present or if nephritis complicates the case. The albumins in question are serum albumin, serum globulin, and nucleo-albumin. The reaction is almost always acid, unless there is a complicating cystitis, which in itself has led to ammoniacal decomposition. The amount of pus is variable, and at times a perfectly clear urine may be voided, owing to temporary blocking of the ureter of the affected side (when the disease is unilateral); this, however, is much more apt to occur in chronic cases. In acute cases the pus is usually abundant. The amount of blood is variable. Frequently it can be demonstrated only on microscopic examination; this should be borne in mind, especially in the diagnosis of renal calculus. Epithelial cells, occurring either singly, with club-shaped or fusiform processes, or arranged in groups and dovetailed together, will also be found; in the latter arrangement they are somewhat, but not absolutely, characteristic. The occurrence of hyaline casts does not necessarily imply any renal involve-

ment in the sense of a nephritis. In calculous cases enormous numbers may appear temporarily in connection with an attack or immediately thereafter, and disappear entirely in a few days. When a complicating nephritis exists, renal epithelial cells, granular casts, epithelial or pus and blood casts will be encountered.

In chronic cases, the amount of urine is frequently increased to from two to three times the normal amount; the specific gravity is normal or diminished and the reaction feebly acid, unless the pyelitis has developed secondarily to a cystitis with ammoniacal decomposition. Sometimes there is a distinct odor of hydrogen sulphide (hydrothionuria), owing to a decomposition of the neutral sulphur bodies by certain microörganisms which have gained access to the urine from without; this may occur independently of ammoniacal decomposition or in association with this. Owing to the presence of pus and sometimes of blood, the urine is more or less turbid, but, as I have already indicated, it is not uncommon to observe periods during which a perfectly clear urine is secreted, owing to a temporary obstruction of the ureter of the affected side or of a diseased calyx, if as sometimes occurs the disease is localized to this extent. In renal tuberculosis the pus appears early and the amount may be quite variable. Sometimes only a few leukocytes are seen, while at others the pus may amount to one-fourth or even one-half of the urine by volume. As a rule, the pyuria is constant, but sometimes cases are seen when for months and even years the urine may be almost clear, owing to improvement in the patient's condition. On the other hand, of course, the passage of apparently normal urine may simply indicate a blockade of the affected side.

In renal tuberculosis hematuria is one of the most important symptoms and not infrequently the first to attract the attention of the patient. The amount is variable; sometimes the bleeding is microscopic, while at others almost pure blood is passed. It is usually intermittent, the periods of bleeding lasting from one to several weeks, the average being three days. Late in the disease it is generally less in amount, but apt to be almost continuous. As a rule, the urine and blood are intimately mixed; clotting may, however, occur either in the bladder or in the pelvis of the kidney. When ammoniacal decomposition has taken place, the pus is transformed into a ropy gelatinous material which escapes as a whole, like a clot of blood, when the urine is poured from one vessel into another. This is sometimes mistaken for mucus, but in reality it is composed of the nucleoproteins, derived from disintegrating leukocytes. In rare cases tumor particles and parasites may be encountered. Epithelial cells from the pelvis of the kidney are usually met with as described above. Albumin, in contradistinction to cystitis, is usually present in fairly large amount (up to 0.3 per cent.), even though the amount of pus be no larger than in a corresponding case of cystitis. Exten-

sive bleeding or nephritis may, of course, further increase the quantity. Cylindruria in chronic pyelitis is exceptional unless nephritis complicates the case.

The Bacteriological Examination of the Urine.—Bacteriological examination of the urine in cases of pyelitis may reveal the presence of a great diversity of organisms. In the acute cases which develop in the course of the various systemic bacterial infections (typhoid fever, erysipelas, pneumonia, ulcerative endocarditis, scarlatinal sepsis, etc.) the corresponding organisms are frequently met with; it may be questioned, indeed, whether pyelitis ever occurs in these diseases without a corresponding bacteriuria. Typhoid bacteriuria is notoriously common in this connection. In calculous pyelitis the cultures are usually negative in early cases; when the disease has persisted for a long time, however, a secondary infection not infrequently develops. The same is true of the tubercular types; ordinary cultures are here commonly negative. As a general rule, the diagnosis is made by the demonstration of the tubercle bacillus in smears made from the sediment. The organisms appear early and are probably always present, but the search for them is frequently very tedious. Small numbers only are found, as a rule, but at times they may be exceedingly numerous; I have seen bunches composed of many hundreds. In doubtful cases one should always resort to the animal experiment, preferably after having destroyed any associated organisms from the bladder with antiformin (which see).

In the mild and often very chronic form of pyelitis which is so frequently seen in women, and in which the general health is but little, if at all, disturbed, the colon bacillus is the usual offending organism. As a rule, it is present in pure culture, but it may be associated with other organisms, notably the proteus vulgaris and staphylococci; in other cases these latter may be met with by themselves.

In a study of 80 cases of pyelitis and pyelonephritis, the majority of which had developed during pregnancy or soon after delivery, Lenhartz found the colon bacillus sixty-six times in pure culture, the paratyphoid bacillus three times, the Bacillus lactis aërogenes twice, proteus vulgaris twice, and Friedländer's pneumobacillus once. In a series of 14 cases studied by Brown (13 women and 1 man) the colon bacillus was found in 8, proteus in 3, a white non-liquefying, but urea-decomposing staphylococcus in 2, while in one instance, of thirty years' duration, no growth was obtained.

Besides the organisms already mentioned, other writers have described the occurrence of the diphtheria bacillus, the gonococcus, the influenza bacillus, the white and yellow sarcina, and still others.

X-ray and Cystoscopic Examination.—Although the site of the lesion, when the disease occurs unilaterally, can often be recognized from the clinical symptoms (pain, swelling, etc.) by *x*-ray or by cysto-

scopic examination, *ureteral catheterization* is sometimes necessary, and should always be practised before excision of a kidney, in order to ascertain the condition of the other organ. Special attention should be paid to the urea content and the microscopic constituents of the specimen. Its functional activity can be further studied by *cryoscopic examination.* Generally speaking, this method of investigation, particularly when applied to the urine, does not furnish information of great value. In the study of renal insufficiency, however, where specimens from each kidney separately are available, or at least one specimen from one kidney and a mixed specimen from the same patient, the method furnishes very satisfactory results. It indicates the location of the disease, or the side which is most damaged (when it occurs bilaterally), more definitely than a quantitative estimation of urea, the specific gravity, and the other usual tests of the urine. Especially interesting are the results which are obtained in unilateral disease, where the other kidney functions normally. Cryoscopic examination of the blood then furnishes normal values, as there is really normal elimination, while a separate examination of the urine from the two sides reveals the diseased kidney. A value of Δ higher than —0.9° C. is abnormal.

Of late the *phenolsulphonephthalein test* has come into use in the study of the permeability of the kidneys, and bids fair to replace the cryoscopic method. It is much more readily carried out and gives very satisfactory results (see technical part).

PYLEPHLEBITIS SUPPURATIVA

Essential Factors.—Marked secondary anemia; hyperleukocytosis with septic factor; bacteriemia; febrile urine with albuminuria and irregular suppurative nephritis; bacteriuria.

The Blood.—*The Red Cells and Hemoglobin.*—Bearing in mind that pylephlebitis is almost always a secondary lesion, the blood picture will be influenced to a certain extent by the nature of the primary malady (appendicular abscess, gastric ulcer, dysentery, cholecystitis, biliary abscess). As the condition, however, is essentially a phase of a general septicemia, it will be readily understood that the development of pylephlebitis, aside from the primary lesion, can produce a more or less intense anemia. In the less acute cases the loss of red cells and hemoglobin may be very extensive, and is very evident already from the appearance of the patient, unless, as may happen, the pallor is obscured to a certain extent by a moderate jaundice. In the more rapidly progressing cases the patient may die before the anemia has become extreme.

The Leukocytes.—The leukocytic picture is typical of a severe septicemia (which see).

Bacteriology.—Bacteriological examination of the blood may reveal the presence of the offending organism. (See Septicemia.)

The Feces.—Exceptionally there is constipation. More common is diarrhea, and it is noteworthy that, owing to the existing stasis, the feces may contain a variable amount of blood.

The Urine.—The urinary picture is typical of an acute febrile process. The urine is scanty, high colored, of high specific gravity, markedly acid, and on standing is apt to deposit an abundant sediment of urates. The urea is said to be markedly diminished. Albuminuria and cylindruria are common, and in some cases a suppurative nephritis may complicate the picture. Since icterus is fairly common, the urine will frequently be found to contain bile pigment. If proper search were made, the offending organism would no doubt be frequently found in the urine.

PYOSALPINX (PELVIC ABSCESS AND PELVIC PERITONITIS)

The Blood.—The blood findings in pyosalpinx, pelvic abscess, and pelvic peritonitis are essentially the same as those noted in appendicitis. In Cabot's series of 76 cases the leukocyte count exceeded 10,000 in 65, and was higher than 20,000 in 26. His highest figure—46,000—was noted in a case of double pyosalpinx, with general peritonitis, ending fatally. When the abscess is well walled off, so that absorption is slight, the count will be correspondingly low.

The hyperleukocytosis is of the neutrophilic type and frequently associated with a diminution or absence of eosinophiles. My impression has been, however, that in many of the gonorrheal cases the eosinophiles persist. Whether or not this point could be utilized in the differentiation of these cases from the ordinary septic cases, remains to be seen.

The *laboratory findings* otherwise are those noted in septicemic conditions in general (which see).

RABIES

(Lyssa; hydrophobia)

The Blood.—Regarding the blood picture in rabies relatively little is known. Courmont and Lesieur found a neutrophilic hyperleukocytosis in 3 cases, which was demonstrable already at a time when nervous symptoms first appeared, and persisted until death. The highest figure observed was 24,800, with 88 per cent. of neutrophiles. In one case the first count, twenty-nine hours before death, was 5000; seven hours later it had risen to 7000; fourteen hours later it was 12,000; and after seven further hours (one hour before death) it was 21,000. It is especially noteworthy that the neutrophile count was 84 per cent. at the time when the total count was only 5000.

According to Franca, there is a mast cell increase, with an average of 2.4 per cent., during the course of the Pasteur treatment. Cabot, on the other hand, reports normal findings in 3 treated cases.

Experiments at complement fixation with rabies-brain as antigen and rabies-serum as amboceptor have led to negative results.

The Urine.—Regarding the condition of the urine no data have been published.

RELAPSING FEVER

(Recurrent fever; typhus recurrens; African and Chinese tick fever)

Essential Factors.—Secondary anemia; irregular hyperleukocytosis of the neutrophilic type; presence of the corresponding spirillum in the blood.

The Blood.—The data bearing on the blood picture of relapsing fever are unfortunately very meager. It appears, however, that a certain degree of *anemia* is a common event, the destruction of the red cells being apparently connected with the occurrence of the paroxysms. At this time also there is *hyperleukocytosis* of the neutrophilic type, which is said to disappear in the intervals; the highest values are observed immediately after the crisis. The degree of increase seems to depend upon the severity of the infection. Laptschinski states that in some instances he found the ratio of the whites to the reds as high as 1 to 37, which would indicate a very considerable increase of the whites, unless, indeed, the reds were especially low, which is not mentioned. Debele, on the other hand, in a series of 19 cases, all of which were benign, notes that while the leukocyte count was higher during the paroxysm than in the interval, there was, in reality, no actual hyperleukocytosis. A leukocytosis up to 10,000 was noted in only 5 cases during the fever, and twice in the apyrexial period. On one occasion, in a second attack, two days before the crisis, the count was 14,400. Among the 18 cases reported by Reiche the total counts varied between 3000 and 12,600.

The Spirillum of Relapsing Fever.—The diagnosis of the disease in sporadic cases must be based upon the demonstration of the corresponding organism in the blood (which see). The number of spirilla which may be found in a drop of blood is variable, being greater during the access of the fever, when twenty or more may be observed in a single field. In some instances they are very scanty, and especially so on the first day of the disease. Subsequently they become more numerous. During the quiescent stage they sometimes occur in the form of rings or of the figure 8. After the crisis they seem to disappear entirely, and their presence during an afebrile period may hence be regarded as indicating a pseudocrisis. During the afebrile periods small, bright, round bodies have been observed in the blood, which, according to some, are spores, while according to others they represent debris of the spirilla.

As in malarial cases, one occasionally meets with leukocytes carrying melanin granules. as well as spirilla in various stages of disintegration.

Culture experiments have been unsatisfactory, though Koch noted an increase in their number at a temperature of 10° to 11° C.

Whether or not the spirillum of Obermeier, which has been observed in European cases, is identical with the organism discovered by Koch in African relapsing fever, and by Hill and others in the corresponding disease in China, has not been ascertained.

The Serum.—Hödlmoser has shown that the blood serum of recurrens is *agglutinating* for the corresponding spirilla; but, as the culture of the organism is practically impossible, the blood of a second case, containing spirilla, must be available for the test.

The Urine.—The data on the urine are so scanty that it is impossible to construct a proper urinary picture. In severe cases, acute hemorrhagic nephritis, with corresponding urinary changes, is relatively frequent. Jarno records that of the 170 cases which he studied at various stages of the disease all presented albumin and frequently granular casts, though this is less common during the third and fourth attacks.

RHEUMATISM (ACUTE ARTICULAR)

Essential Factors.—Secondary chlorotic anemia with hyperleukocytosis of the neutrophilic type; early return of the eosinophiles with epicritic hypereosinophilia; irregular bacteriemia; tendency to albuminuria; increased elimination of uric acid.

The Blood.—*The Red Cells and Hemoglobin.*—A certain degree of anemia develops in all cases of acute articular rheumatism, but in some cases it does not become especially evident until convalescence begins. Generally speaking, it is proportionate to the intensity of the disease. In the majority of cases the loss in hemoglobin equals or slightly exceeds that of the red cells, so that the color index frequently is a little below 1; exceptionally, it is higher. In McCrae's series of 33 cases the average red count was 4,636,000, and the corresponding hemoglobin value 73.4; Cabot's figures were 4,400,000 and 67 per cent., and Da Costa's, 3,686,648, with 63 per cent. In the Hopkins series of 77 cases, 45 gave counts between 4,000,000 and 5,000,000, and 14 such of 5,000,000 and over. Some of these high counts, no doubt, are only relatively high, and dependent upon excessive loss of body fluid by sweating. Occasionally the disease produces very severe anemia, the corpuscles falling to 1,000,000 to 2,000,000 and the hemoglobin to 30 per cent. and lower. Normoblasts, according to Türck, may be found in 25 per cent. of the cases.

The Leukocytes.—The leukocytes are increased in all acute cases, the height of the leukocytosis being roughly an index of the severity of the disease. The highest values are found in association with complicating conditions, such as endocarditis, pericarditis, pneumonia, hyperpyrexia, etc. In the Hopkins series of 81 cases, mentioned by Emerson, the usual values were between 10,000 and 15,000. In McCrae's analysis it was 12,370; in Cabot's, 16,800; and in DaCosta's, 12,218; exceptionally the leukocytosis reaches 30,000 and even 40,000. The differential count shows that the leukocytosis is of the neutrophilic type. Early in the disease the septic factor is usually pronounced, but after a short while the eosinophiles return to normal, notwithstanding the active progress of the disease, and during convalescence an epicritic eosinophilia is common, then the values may reach 13 per cent.

The Plaques.—The plaques are much increased during the febrile period.

The Fibrin.—The fibrin is much increased during the active stage of the disease, a factor to which the tendency to the formation of vegetations upon the valves and of clots in the heart and arteries is to a great extent due. The coagulation time is, if changed at all, increased.

The Alkalinity.—Regarding the alkalinity of the blood, trustworthy data are not available; the *uric acid* content is not increased, while *lactic acid* is said to be present in excess.

The Bacteriological Examination.—Bacteriological examination is almost always negative. Some writers, however, have found streptococci, staphylococci, diplococci, and certain anaërobic bacilli; but the relation of these organisms to the disease is not altogether beyond question. In a series of 10 cases of mild acute endocarditis following what clinically appeared to be typical articular rheumatism, Libman could demonstrate attenuated streptococci and diplococci during extended periods of time.

The Urine.—The urine shows two general characteristics of a febrile urine. While the disease is active and perspiration copious it is highly colored, strongly acid, and of a high specific gravity; at the same time there is a marked tendency to the deposition of urates and uric acid. Albumin is present in about 40 per cent. of the cases. Its appearance is usually temporary, and unattended by any evidences of renal inflammation; rheumatismal nephritis is esceptionally observed. Sugar is absent. Indican is frequently abundant. The urea is quite variable, the average elimination being about 25 grams in the twenty-four hours, with 20 grams and 40 grams as maximal and minimal values. The uric acid is said to be always increased, the average being 1.5 grams. The chlorides are usually much diminished, the average being about 5 grams.

RICKETS

Essential Factors.—Chlorotic anemia; hyperleukocytosis; lymphocytosis.

The Blood.—*The Red Cells and Hemoglobin.*—While cases of rickets have been described without any marked diminution of the red cells and hemoglobin, anemia is unquestionably the rule. It is of the chlorotic type, but the red cells are usually also diminished quite materially. Hock and Schlesinger's average in a large number of cases was 2,500,000. Severe anemia is usually only met with in complicated cases, but it is noteworthy that it may occur even when no manifest cause for increased cell destruction is apparent. In a case reported by von Jaksch, the red cells fell from 1,600,000 to 750,000 within three months, and Luzet mentions a drop from 2,110,000 to 1,596,000 in three weeks. Such cases, however, are exceptional; as a rule, the oligocythemia develops more gradually. While the color index is usually diminished, an increase may be observed in the severe types of the disease. True pernicious anemia, however, bears no apparent relation to rickets.

In markedly anemic cases *normoblasts* may be seen in large numbers; polychromatophilia is then also common.

The Leukocytes.—In most cases of moderate severity the leukocytes do not appear to be especially increased. In the series reported by Morse, in which the average age was one year, all the counts but three were lower than 16,000. In the severe cases, on the other hand, there is a hyperleukocytosis of varying degree. In many the figures do not exceed 30,000, but in some a blood condition develops which is scarcely to be distinguished from the anemia of v. Jaksch (anemia infantum pseudoleukemica).

Compared with the blood of adults, rickety children usually show a higher percentage of lymphocytes; but this is often not excessive when compared with the values obtained in healthy children of the same age. In others, however, there is unquestionably a pathological increase. The eosinophiles, in most cases, are not increased, but in a few abnormally high values have been reported (16 to 20 per cent.). At times a few myelocytes may be seen.

The Urine.—The urine shows no changes which are characteristic.

SARCOMATOSIS

Essential Factors.—Chlorotic anemia with relative polycythemia; hyperleukocytosis, usually of the neutrophilic type, with a tendency to normal eosinophile values; occasional lymphocytosis; no increase in the amount of blood sugar; presence of protective ferments in the blood; presence of melanogen in the urine in melanotic cases.

The Blood.—*The Red Cells and Hemoglobin.*—As in carcinomatosis so also in sarcomatosis, anemia of the chlorotic type is a constant feature which develops sooner or later in every case. But here as there the laboratory findings may not correspond to the actual condition, as a concentration of the blood may obscure the real anemia. An analysis of the findings recorded by Da Costa, Cunliffe, and Cabot (55 cases) gives 4,220,000 as average red count, with 2,240, 000 as minimal and 6,200,000 as maximal values. Still lower values may, however, be observed; Hayem thus records an instance of osteosarcoma with only 663,400 cells. The loss of hemoglobin exceeds the loss of red cells, and is greater on an average than in carcinoma. In Cabot's series of 16 cases the average figure was 59 per cent.; not infrequently it falls to 30 per cent., and even lower. Rieder records a case in which it reached 6 per cent., the lowest figure that I have met with in the entire hematological literature.

Morphological examination shows the usual changes of a secondary anemia, viz., pale corpuscles, a variable grade of poikilocytosis and some changes in size. Stiple cells are scanty. Erythroblasts, chiefly normoblasts, may be met with, but are less frequent than in carcinoma.

The Leukocytes.—The leukocytes are increased in the majority of cases. Of 48 cases mentioned by Cabot, hyperleukocytosis was noted in 32, the values ranging between 9800 and 98,000. The highest average value is seen in melanotic sarcoma—25,100; lymphosarcoma follows with 20,000, and osteosarcoma with 17,000. In myelomatosis the recorded figures vary between 4500 and 40,000. Sometimes a remarkable increase of the cells is observed during the progress of a case. In one instance of melanotic sarcoma the count rose from 17,000 to 55,400 in barely two months. The hyperleukocytosis is usually due to an increase of the neutrophiles, and here, as in carcinoma, this increase is frequently associated with a persistence of eosinophiles in normal numbers; at times a marked hypereosinophilia has been noted, which is not always accounted for by metastases to the bone marrow. Neutrophilic myelocytes in moderate numbers are frequently observed in cases showing hyperleukocytosis. At times the small mononuclear leukocytes are increased in sarcoma. Such cases usually show a hyperleukocytosis, but sometimes a lymphocytosis is found even though the total number of leukocytes is not increased, and occasionally a polynucleosis may occur under similar conditions.

Serology.—The serology of sarcomatosis has thus far received very little attention. In one case mentioned by Brieger and Trebing the antitryptic content was increased. Two cases which I examined showed normal values.

On the occurrence of isohemolysins no data are available in the case of man, but to judge from Weil's findings in lymphosarcomatosis in dogs, it would not be surprising if they were present.

Protective ferments have been demonstrated in the blood of the few cases in which they were sought for, and it is noteworthy that the patient's serum reacted only with sarcomatous and not with carcinomatous tissue.

The Chemical Examination.—Chemical examination, according to Freund and Trinkler, shows no increase in the amount of blood sugar in sarcoma, while in carcinoma this is said to be increased. (See Cancer.)

The Gastric Juice.—There are no data available to decide whether in sarcomatosis, as in cancer of organs other than the stomach, the formation of hydrochloric acid is impaired.

The Sputum.—The sputum in sarcoma involving the lungs shows the same general characteristics which are noted in carcinoma. Here, as there, particles of the tumor are occasionally found.

The Urine.—The available data are insufficient to construct an adequate urinary picture of sarcomatosis; it appears, however, that the general changes are the same as those which are seen in carcinoma.

In melanotic tumors the urine frequently contains melanogen which is transformed into melanin on exposure to the air. Such urines present a normal color when first voided, but on standing they darken, and may finally turn black. This symptom, however, is not pathognomonic, as it may be absent in melanotic cases and present in non-cancerous cases. The demonstration that the darkening of the urine is due to melanin and not to other substances is, nevertheless, a strong point in favor of melanotic sarcoma.

SARCOSPORIDIASIS

In one case, described by Darling, the *leukocyte count* during the first week rose from 5400 to 16,000. A single examination in the third week showed a leukocytosis of 15,600 with 25 per cent. of small mononuclears, 20 per cent. of large mononuclears (including 6 per cent. of transitionals), and 55 per cent. of polymorphonuclears. In the seventh week, a count of 12,100 is recorded, with practically the same differential formula, plus 3 per cent. of eosinophiles and 1 per cent. of mast cells. About a fortnight later, the total count was 8500, with 58 per cent. of mononuclear elements (of which 42.5 per cent. were small mononuclears), 39 per cent. of neutrophiles, 2.5 per cent. of eosinophiles, and 0.5 per cent. of mast cells.

The *red count* (no doubt owing to blood concentration) was not diminished.

The corresponding organism—the *sarcocystis hominis*—was found in sections of the biceps muscle.

Darling viewed the condition as an accidental complication in the course of typhoid fever, evidently basing the diagnosis of the latter disease on a single positive Widal reaction in the fifth week (other examinations negative) and a positive diazo reaction in the fourth.

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MIASIS JAPONICA

HELMINTHIASIS)

SCURVY

Essential Factors.—Chlorotic anemia; hyperleukocytosis; diminution of the plaques; normal coagulation time.

The Blood.—*The Red Cells and Hemoglobin.*—In practially all cases there is anemia of the chlorotic type, the extent of which is, in a general way, proportionate to the intensity of the morbid process. As in the other hemorrhagic diseases, there is a remarkably active blood regeneration, however, so that extensive hemorrhages are compensated in a fairly brief period of time. In a case described by Bouchut, in which as the result of severe bleeding from the nose the red count had dropped to 557,875, it had risen to 3,627,000 three months later. Uskow and Hayem gives counts varying between 3,500,000 and 4,700,000 in the lighter cases.

The hemoglobin values are lower than the corresponding red counts. Hales White found the hemoglobin reduced to 20 per cent. and the red cells to 45 per cent., giving a color index of 0.44.

The morphological changes are not in any way characteristic, but are essentially those of a secondary anemia.

The Leukocytes.—Hyperleukocytosis is a common symptom and, no doubt, largely dependent upon complicating factors (inflammatory disturbances and hemorrhages). Uskow gives values ranging between 20,000 and 47,000. Stengel mentions an instance with 40,000 (post-hemorrhagic) and lymphocytosis.

Lütten, Ewing and others have reported cases with no leukocytic increase. Regarding the leukocytic formula, there are no data beyond the findings of lymphocytosis in the one case just mentioned.

The Plaques.—The plaques are much diminished, and may even be absent in severe cases.

The Coagulation.—While there is a strong tendency to hemorrhage in scurvy, the coagulation time is not necessarily diminished. In 8 cases, mentioned by Lamb, it varied between one and one-quarter minutes, with an average of about three and one-half.

The Alkalinity.—The alkalinity according to the same writer, and in contradistinction to Wright, is not diminished.

Hemorrhages.—Hemorrhages from the mucous surfaces and from the internal organs, leading to nose bleed, hematemesis, melena, hematuria, etc., are seen especially in the severer cases, while subcutaneous and intramuscular hemorrhages are common events.

The Urine.—A detailed study of scorbutic urine has apparently not been made. Albuminuria occurs essentially in the severe cases and in some instances acute nephritis, with the corresponding urinary picture, has been observed.

SEPTICEMIA

The following account of the clinical laboratory findings in septicemia is based upon the study of infections with the common pus organisms, viz., staphylococci, streptococci, and pneumococci. Other types of septic infections are considered under their respective headings.

Essential Factors.—Severe secondary anemia; hyperleukocytosis with septic factor; bacteriemia; bacteriuria.

The Blood.—*The Red Cells and Hemoglobin.*—In all cases of staphylococcus and streptococcus septicemia there is a more or less extensive destruction of red corpuscles, which usually sets in quite early and progresses with great rapidity. There is no other pathological condition in fact, with the possible exception of malaria, in which so extensive an anemia may develop in so short a time. According to Hayem, the usual loss from septic fever amounts to from 200,000 to 1,000,000 corpuscles per week. After the anemia has reached a certain grade, however, further loss occurs more slowly. Average figures, denoting the extent of anemia observed in a series of cases, serve no useful purpose, as it is difficult to compare individual cases with one another. Suffice it to say that the number of corpuscles not infrequently drops below the 2,000,000 mark, if the infection is severe and the patient's resistance sufficiently strong so that death does not take place early in the disease. In a case of lumbar abscess which had existed for about six months I obtained a red count of 1,025,000. Cabot mentions a suppurating fibroid with 1,800,000, and Ewing one of chronic empyema with the same number. The most extreme grades of anemia, however, are noted in cases of puerperal septicemia; in some of these a progressive corpuscular destruction can be demonstrated from day to day, and even from the morning to the evening of the same day. In a case of this kind, Da Costa noted a count of 730,000, and Grawitz mentions an instance, associated with profuse hemorrhages in which the red cells fell to 300,000 within twenty-four hours. Occasionally, but not often, cases of septicemia are met with in which the question of pernicious anemia may enter into consideration. This occurred in two cases of gonorrheal endocarditis observed by Osler. (See Gonococcus Infections.)

So long as fever exists the red count in all cases of septicemia probably does not express the actual state of anemia, owing to concentration of the blood, while this becomes more manifest as the patient begins to improve.

The loss of hemoglobin at least equals the loss of red cells, and usually exceeds it to a slight extent, so that a somewhat lowered color index is common. An increased index is very rare. In the lumbar abscess case, above referred to, the hemoglobin had fallen to 17 per cent.

Morphological examination in a well-marked case of septic anemia gives results which are somewhat characteristic. The pallor of the individual red cell attracts attention at once. Deviations in size and form are not especially marked, but there is evident a certain loss of elasticity, so that many of the cells appear creased and wrinkled. In some cases the process of hemocytolysis is easily demonstrable on microscopic examination; some of the red cells then show a frayed margin with a surrounding area of diffused hemoglobin, and on careful examination blood shadows may also be found. A certain grade of polychromasia is common, and as many of the red cells which present this are manifestly not normal, it is not surprising that many writers have come to look upon this appearance as a degenerative phenomenon. Stiple cells are not numerous. Normoblasts in small numbers may be found in all cases.

In the wet preparation the tendency to *money-roll formation* is found much diminished. In markedly anemic cases the *formation of fibrin* is also below par, while early in the disease it is frequently increased.

The Leukocytes.—The leukocytes are increased in all cases of septic infection, unless the disease is unusually mild or so severe that no defensive reaction of moment occurs. The deciding factors are thus the intensity of the infection and the power of resistance on the part of the individual, given as common premises the occurrence of active resorption from the seat of infection. As the opportunity for resorption will be greater in some tissues than in others, it follows that the tendency to hyperleukocytosis also will vary with the location of the infection. The same considerations apply to the extent of the leukocytic increase. In some cases this does not greatly exceed the maximal normal limit of 10,000, while in others the count may go to 50,000 or higher. In the psoas abscess case, above referred to, I counted 72,000 per c.mm.; Cabot found 77,500 in a case of puerperal septicemia. In the majority of cases, however, the figures vary between 15,000 and 30,000. In the lightning cases, on the other hand, *i. e.*, in those in which the patient is overwhelmed with the intoxication from the very start, no increase is met with; the numbers are normal, or there may be leukopenia. Where hyperleukocytosis exists it will be found that the increase is referable to the polynuclear neutrophiles, the relative number of which is generally proportionate to the total number, usually ranging between 80 and 90 per cent. Higher values indicate an especially severe infection; in one case of this order I found 99 per cent. Coincidently with the neutrophilic increase there is a decrease or absence of eosinophiles. To the association of these two factors I have applied the term "septic factor," and I would emphasize that *the septic factor is met with in all cases of septicemia referable to the common pus organisms, and is demonstrable even in those cases which are unattended by hyper-*

leukocytosis. This fact sufficiently indicates the great value of the differential count, and it will be readily understood why I have insisted upon its importance for so many years, at the expense, if need be, of the absolute count. Both counts together give the clearest picture of the intensity and character of the infection, as also of the defensive reaction of the body, but *when one count only is to be made, this one should be the differential. The physician at large has been very slow to learn this lession—one of the most important that is taught in the clinical laboratory.* If in a clinically manifest case of septicemia, the eosinophiles are found in normal or increased numbers, it may be taken for granted, either that the patient is improving (epicritic eosinophilia), or that some other factor is simultaneously active, which in itself would lead to eosinophilia.

Neutrophilic myelocytes in small numbers may be demonstrated in any case of septicemia, which is associated with hyperleukocytosis. A study of the karyomorphism of the neutrophiles, moreover, will show that the vast majority of the cells belong to Classes I and II, with single or two lobes, while the other cells, in the sense of Arneth, are much diminished in number or absent.

Important to note is the fact that *iodophilia* of the neutrophiles is observed in all cases of septicemia—a factor upon which some writers have laid much stress.

The small and large mononuclear leukocytes are both relatively and absolutely diminished. The same usually holds good for the mast cells, statements to the contrary notwithstanding. A few phlogocytes, on the other hand, may be present.

Serology.—The diagnosis of the different types of septicemia on the basis of the presence of agglutinins in the serum has been repeatedly attempted, but does not seem practicable at present. The same is true for the opsonic diagnosis of these conditions.

Hemoglobinemia.—This is demonstrable by the microscope in many cases. The naked eye appearance of the serum frequently suggests the same, but this inference is scarcely justifiable without a spectroscopic examination, as reddish sera are not infrequently observed in conditions where active hemolysis is probably not taking place.

The Solids.—The solids, both of the whole blood and the serum, and notably the albumins, may be much reduced; in fatal cases Roscher found a drop to 15 per cent. (calculated for the whole blood). In the case of the serum the loss of native albumins may be obscured by the hemoglobinemia.

Bacteriology.—The bacteriological findings in cases of septicemia will depend, to a large extent, upon the experience of the individual investigator in this particular direction. In this way only is it possible to account for the widely different results which have been recorded by different observers. The earlier records bearing on this

point have relatively little value, as the technique employed was not always satisfactory. The most extensive *recent* studies are those of Libman. According to this investigator Streptococcus bacteriemia is more common than Staphylococcus bacteriemia. In his series of about 1000 bacteriological blood examinations he found the former in 58 and the latter in 28 cases. Pneumococci are more rarely met with. Among the streptococcus cases some were instances of terminal infections, or infections arising from the tonsils, the ears, and mastoid processes, while in others infection was due to wounds, and in still others cryptogenetic. Some cases were characterized by joint or bone lesions. Endocarditis was frequent (which see). One was a case of erythema nodosum. In a recent paper, Libman and Celler have reported their findings in 75 cases of otitis media or mastoid disease without sinus thrombosis or meningitis, and in all these cases the blood was sterile. Positive results were obtained early in those otitis cases in which one or the other of these complications existed. (See Otitis.)

Of the staphylococcus cases, a number were instances of osteomyelitis, some were secondary to furuncles or cellulitis, others were cryptogenetic, and two referable to postpartum infection (rare). All three were aureus cases. The only positive albus cases (not referable to contamination) were obtained within forty-eight hours before death, and Libman looks upon these as agonal invasions. The Staphylococcus citreus was isolated once in a case of osteomyelitis.

Pneumococci, apart from pneumonia, were only met with in four cases of Libman's series. Twice there was an acute endocarditis of unknown source, once there was an infection between two toes, and once there was a suppurating ethmoiditis and frontal sinusitis with abscess. Other observers have found the organism in cases of biliary abscess at the time of the chill, in suppurative oöphoritis, in peritonitis, etc.

Hektoen has pointed out that in scarlatina streptococci may be found in the blood during life in at least 18 per cent. of all cases. I append his conclusions: Streptococci may occasionally be found in the blood of scarlet fever cases that run a short, mild, and uncomplicated clinical course. They occur with relatively greater frequency in the more severe and protracted cases of the disease, in which there may also develop local complications and clinical signs of general infection, such as joint inflammations; but even in the grave cases of this kind spontaneous recovery may take place. In fatal cases streptococci may not be demonstrable. The theory that scarlet fever is a streptococcus disease thus does not seem to receive direct support from these investigations.

In diphtheria, measles, and smallpox, infections with streptococci are also not uncommon. Other organisms may, however, also be met

with, such as the various staphylococci, and quite commonly also, according to Jehle, the bacillus of influenza.

While positive findings are often of the greatest value in the diagnosis of obscure cases of septicemia, negative results call for great caution in their interpretation. Every case must be judged by itself, and repeated cultures made in doubtful cases.

The number of organisms which may be found in the blood at one examination is quite variable. On the one hand, but one plate or flask out of several may show any growth, and then only after several days; while, on the other hand, the number of organisms may be quite large. Cole has reported a case of Streptococcus septicemia in which the number of organisms amounted to 3642 per cubic centimeter of blood six days before death. I have seen a case of meningococcus septicemia in which the organisms numbered 7,380,000 per cubic centimeter just before death.

The time before death at which organisms may be found in the blood is also quite variable; sometimes they may be demonstrable a month before, in other cases only a day or two before, the fatal issue.

In cases where the focus of infection can be removed by operation it is often remarkable to see with what rapidity the organisms disappear. Libman states that in a case of sinus thrombosis complicating otitis media he has seen the count drop from 245 to zero within twenty-four to forty-eight hours, after ligation of the jugular vein. In another instance of a similar nature the bacteriemia disappeared in less than four hours.

As regards the general prognosis of those cases in which pyogenic bacteria are found in the blood, this is usually unfavorable, owing to the possible formation of secondary foci of infection. Recoveries, however, are possible. Each individual case must be judged separately. In Libman's series of streptococcus bacteriemia there were 6 recoveries (or 11 per cent.); of the 28 cases of staphylococcemia, 8 recovered (or nearly 29 per cent.), and of his 4 pneumococcus cases, 1 recovered. In Bertelsmann's series of 48 cases of surgical bacteriemia, 21 recovered, (or 43 per cent.); among these there were 28 streptococcus cases, with 19 recoveries, and 13 staphylococcus cases, with 4 recoveries. In Lenhartz's series of 77 medical cases (including several postpartum infections) there were 17 recoveries; among these there were 47 streptococcus cases, with 6 recoveries, and 13 staphylococcus cases, with 1 recovery.[1] Of 55 cases of infection with Streptococcus viridans in which the organism was found in the blood, recorded by Warren and Herwick, 25 ended fatally, 3 are noted as unimproved, 2 as improved, and 10 as cured (18.1 per cent.). Of 31 infections with Streptococcus hemolyticus 7 recovered (22.5

[1] For many interesting details, the reader is referred to Libman's principal papers, which are published in the Johns Hopkins Hospital Bulletin and the American Journal of the Medical Sciences, September, 1909.

per cent.), while 21 died. Of 39 infections with Staphylococcus aureus with positive blood cultures 14 recovered (35.8 per cent.) and 22 died. Of 10 pneumococcus cases 2 recovered (20 per cent.) and 6 died.

The Urine.—The urine shows no special characteristics which could be utilized for diagnostic purposes beyond the fairly frequent presence of the offending microörganisms (bacteriuria). Pyogenic cocci are especially prone to settle in the kidneys and there give rise to focal inflammation; but even in the absence of such lesions they are frequently found in the urine. In all forms of infectious nephritis, an abundant elimination of the corresponding bacteria may generally be observed. Von Jaksch is authority for the statement that in erysipelas, the bacteriuria and nephritis disappear with the cessation of the disease, and in various suppurative processes the specific bacteria disappear from the urine within twenty-four to forty-eight hours after evacuation of the pus. In pneumococcus infections I have repeatedly found pneumococci, and in scarlatina streptococci have been observed in a large percentage of cases; the urine was then usually albuminous. To what extent the study of bacteriuria could replace the bacteriological examination of the blood would be an interesting theme for future research.

The urine otherwise shows the same general features which are common to most acute febrile diseases. The color and the specific gravity are high, the reaction strongly acid, and the volume more or less reduced. The mineral constituents are diminished, while metabolic experiments show an increased nitrogenous katabolism. The presence of albumin is common, even though no actual nephritis exists; when this complicates the clinical picture the albuminuria is more intense, and leukocytes, red corpuscles, and all types of casts may be encountered in large numbers. (See Infectious Nephritis.) The diazo reaction is uncommon.

SIDEROSIS

In siderosis the sputum presents a brownish-black color and contains cells inclosing particles of ferric oxide. These may be readily recognized by treating with a drop of ammonium sulphide or potassium ferrocyanide solution in the presence of hydrochloric acid, when a black color, on the one hand, or a blue color, on the other, is obtained in the presence of iron.

SKIN DISEASES

Essential Factors.—General tendency to hypereosinophilia.

The Blood.—Regarding the behavior of the *red cells and hemoglobin* in the various skin diseases there are no satisfactory data. Interest in the past has centred in the frequent occurrence of *hypereosinophilia* of greater or less extent, and it appears from the collected

observations that the degree of increase, other things being equal, depends very largely, though not exclusively, upon the extent of the local process, and upon the severity of the disease. Light cases may show values which are practically normal.

The findings in the more important skin lesions are here briefly considered:

Pemphigus Foliaceus.—Hypereosinophilia is here unquestionably one of the most constant symptoms. As its extent, however, as Zappert originally showed, runs a course parallel to the extent and severity of the disease, it will be readily understood that no increase of the eosinophiles may be observed in very recent cases or during periods of remission. In the 6 cases reported by Zappert, the total leukocyte count ranged between 4590 and 16,400, and the eosinophiles between 1.6 and 33 per cent. Drysdale mentions a patient in whom the eosinophiles rose to 60 per cent. twenty-four hours after the eruption of the vesicles, and declined to 8 per cent. within the next ten days.

Corresponding results have been found in *pemphigus vegetans*, but here also normal values may at times be encountered.

Dermatitis Herpetiformis (Dühring).—The eosinophilia ranges from 12 to 44 per cent. In one case reported by Brown, which had existed for twenty-seven years, the white count during a period of three months varied between 9000 and 14,000, and the eosinophiles between 29.2 to 44.3 per cent. The red count was not diminished. Exceptionally there is no hypereosinophilia, though it has been suggested that some cases of this type may not have been actual cases of the disease in question.

Brown noted 9.7 per cent. of eosinophiles in a case of *bullous epidermolysis hereditaria.*

Herpes Zoster.—According to Sabrazès and Mathes there is an initial hyperleukocytosis during the first two or three days (11,000 to 17,900) which is followed by a decline during the period of desiccation and a slight subsequent rise. Normal values are reached again by the end of the second week. The hyperleukocytosis is of the neutrophilic type, but associated with normal or increased eosinophile values; the highest number of the latter (8 to 20 per cent.) is reached during the stage of desquamation.

Brown has observed a leukocytosis of 10,700, with 25.2 per cent. of eosinophiles in a case of *herpes tonsurans.*

Prurigo.—According to Peter, hypereosinophilia appears early and is constant (10 per cent. or more.).

Eczema.—In cases presenting a well-localized eczema the blood examination shows no abnormalities. In generalized cases, however, hypereosinophilia seems to be common (4.07 to 45 per cent.); coincidently there is a more or less extensive absolute hyperleukocytosis (up to 20,000). Exceptions also occur. Cabot mentions

one acute case with a leukocyte count of 15,000, with no relative increase of the eosinophiles.

Impetigo Contagiosa.—Data are available in only two cases (K. Meyer). The total leukocyte counts were 18,500 and 8400 with 8.2 and 4.9 per cent. of eosinophiles respectively. The disease had existed for a fortnight in the first, and a week in the second case.

Psoriasis.—Hypereosinophilia of moderate extent (up to 17 per cent.) is common, but there are many cases in which the values scarcely exceed the normal. Occasionally there is an increase during treatment.

Lichen Ruber Planus.—Leredde has noted 6 per cent. in one case, while Zappert found 3.28 in another.

Erythema Multiforme.—In Cabot's series of 7 cases the total leukocyte count ranged from 6150 to 19,700, being above 10,000 in three. No increase of the eosinophiles was noted. Leredde and Sabrazès are the only writers who mention values higher than normal, viz., 6 to 6.9 per cent.

Urticaria.—Urticaria cases show a very irregular behavior. In some there is no deviation from the normal, while in others there may be hypereosinophilia of high grade. Lazarus mentions an acute case with extensive distribution with 60 per cent.

Ichthyosis and Scleroderma.—In one case of ichthyosis Müller and Rieder found 1.3 per cent. of eosinophiles, and in three cases of scleroderma Zappert found 9.47, 7.71, and 4.51 per cent. respectively. In cases of scleroderma pigmentosum, Okamura observed a leukocytosis averaging 40,000 cells with a moderate hypereosinophilia in two.

Mycosis Fungoides and Lupus.—In mycosis fungoides the values may be diminished, normal, or increased (up to 37 per cent.). The same is true of lupus (1.2 to 12.24 per cent.).

Ki-Mo.—Sabrazès and Mathes have described hypereosinophilia in connection with a disease termed Ki-Mo, which occurs in Tonkin and Laos.

Toxic Dermatoses.—Among the dermatoses of toxic origin, quicksilver intoxication is especially apt to cause an increase of the eosinophiles (up to 31.5 per cent.); picric acid and benzin may be similarly active (up to 25 per cent.). Leredde and Poutrier have observed hypereosinophilia in association with a skin eruption following the ingestion of antipyrin.

SPLENIC ANEMIA

(Banti's disease)

Essential Factors.—Chlorotic anemia; marked tendency to leukopenia; usually no change in the leukocytic formula.

The Blood.—*The Red Cells and Hemoglobin.*—At the time when the patient first comes under observation there is already a well-developed

anemia. As in chlorosis, the oligochromemia exceeds the oligocythemia, so that a low color index is obtained. Here, as there, the loss of corpuscles is relatively slight. Of the 41 cases collected by Osler, the average count was 3,425,000, the lowest 2,187,000, and the highest 5,200,000. The corresponding hemoglobin values were 47, 23, and 60 per cent.; this would correspond to a color index between 0.52 and 0.57. This picture continues throughout the greater portion of the course of the disease. In the later stages, however, the corpuscular anemia more nearly approaches the oligochromemia, with a corresponding rise of the index.

Morphological examination in most cases shows no essential deviations, beyond the pallor of the red cells, excepting later in the disease, when poikilocytosis, anisocytosis, and polychromatophilia may become prominent features. Granular degeneration plays no role. Nucleated red cells are usually scarce, but exceptionally they may be quite numerous. In one of the Hopkins cases, with a reduction of the red cells to 27.6 per cent. and of the hemoglobin to 20 per cent., 75 erythroblasts were encountered while counting 400 leukocytes; of these, 21 were normoblasts, 19 megaloblasts, and 35 unclassified forms.

The Leukocytes.—While normal numbers may be met with at successive examinations of an individual case, there is, on the whole, a distinct tendency to leukopenia. Osler's average was 4520, the individual counts ranging from 2000 to 12,497. The higher values are obtained after profuse hemorrhages, or as the result of a terminal infection. Many cases, at the first examination, give counts between 2000 and 4000. Occasionally the number may fall below 1000. Osler mentions a case of Vickery's in which only 650 to 700 leukocytes were counted per c.mm., and one of Peabody's with 800 cells.

The differential count usually shows no material deviation from the normal. In some cases a combined or individual lymphocytosis and splenocytosis have been observed. This, however, is by no means constant, and is more apt to be seen in the especially anemic cases. A few myelocytes are not uncommon.

The Plaques.—The plaques are not increased.

Other Factors.—Regarding the coagulability, alkalinity, specific gravity, and general chemistry of the blood in splenic anemia, nothing definite is known. During the terminal stage of the disease, with hypertrophic cirrhosis of the liver (Banti's disease), the serum is markedly bile-tinged.

The Urine.—The urine shows no characteristic changes. The quantity is normal. Sugar is absent, but in one case Sippy was able to produce a digestive glucosuria by the administration of a "moderate quantity" of glucose. A trace of albumin may be found, but there are no evidences of any extensive degenerative changes in the kidneys. Stein claims to have found a large amount of nucleohiston in a case which was clinically diagnosticated as splenic pseudoleukemia. In the Banti stage bile pigment is present.

SPOTTED FEVER (MONTANA)

The Blood.—Moderate anemia of the chlorotic type is observed in nearly all cases. The leukocytes may show a mild increase (12,000 to 13,000) and occasionally a slight large mononucleosis.

The supposition that the disease is caused by a hematozoan parasite, the *piroplasma hominis*, has not been proved. I have studied the blood of several cases which were placed at my disposal by medical friends, but was unable to find such structures as have been described by Wilson, Chowning, and Anderson. Craig and Stiles express themselves in a similar manner.

The Urine.—Regarding the urinary condition there are no data.

SPRUE

In well-pronounced cases of sprue a monilia may be demonstrated in scrapings from the base of the tongue lesions, as well as in the feces which differs from the M. albicans of thrush, and is regarded by several observers as the cause of the malady (Bahr, Ashford).

There is a tendency not only to achylia gastrica, but also to pancreatic achylia, which may persist even after the original moniliasis of the intestinal tract has disappeared.

STOMACH (DILATATION OF)

Essential Factors.—Actual anemia with relative polycythemia; high grade of motor insufficiency; variable secretory changes; fermentation and gas production; oliguria; deficiency of chlorides; occasional acetonuria and diaceturia.

The Blood.—*The Red Cells and Hemoglobin.*—The blood picture in dilatation of the stomach will depend to a great extent upon the nature of the underlying condition. But aside from this there can be no doubt that the dilatation *per se*, through the consequent impairment of the patient's digestion and deficient absorption of water, can give rise to material changes in the condition of the blood. In many cases of this kind, however, the actual anemia is largely obscured, so far as numerical findings go, by a considerable concentration of the blood. As a consequence, there is frequently a lack of all proportion between the manifest mummification of the patient and the red count. In such cases a proper idea of the degree of anemia could manifestly only be obtained, if it were possible to estimate the total bulk of the blood and to calculate the corresponding count, for the total volume brought up to what it would be for the individual in question. The normal or even increased red counts in long continued cases of dilatation hence convey an erroneous idea, unless controlled by the patient's body weight. The same holds good, though

to a somewhat less extent, for the hemoglobin content; usually this will be found lower than what would correspond to the associated red count. Markedly low figures (2,250,000 to 3,300,000) are thus the exception both for the red cells and hemoglobin.

The Leukocytes.—The leukocytes also are frequently found to be relatively increased. That there is no absolute increase is manifest from the corresponding differential count which shows normal values of the various forms, with possibly an occasional tendency to a relative lymphocytosis.

The Stomach Contents.—In cases of gastric dilatation of high grade, especially when referable to pyloric stenosis, vomiting is a common symptom. This usually occurs a number of hours after eating. The amount is large, and commonly there are found remnants which have been eaten the day before or still earlier. The appearance of the material depends to a great extent upon the condition of the secretory mechanism. When this is much impaired, as in cancerous stenosis of the pylorus, there will be much undigested food (large pieces of meat and bread), while in cases of hyperacidity and hypersecretion, protein digestion is well advanced, but trisedimentation a common symptom. Blood is occasionally present; more particularly in cases of cancer or ulcer.

The Motility.—The impaired motility, aside from the vomiting of food material that has been consumed long before the last meal, is manifested by the delayed excretion of salicyluric acid, following the administration of salol (test of Ewald and Sievers, which see), or of iodine after the ingestion of iodipin (test of Winternitz). The same information, of course, may be obtained more satisfactorily by giving the patient an evening meal of known composition (meat, bread and butter, and tea) and then washing out the stomach on the following morning, when the presence of food remnants indicates a very considerable degree of motor insufficiency.

The Chemical Findings.—The chemical findings in gastric dilatation are variable. In cases of cancer there is usually continued absence of free hydrochloric acid, and presence of large amounts of lactic acids, while in benign pyloric stenosis hyperchlorhydria is the rule. In cases of stenosis of the duodenum bile is continuously present, and in such an event the peptic activity is apt to be lost; small amounts of bile, however, seem to be innocuous in this respect.

Fermentative Processes and Gas Production.—In many cases of gastric dilatation fermentative processes and gas production are common events. In the latter event the vomited material is markedly frothy. Lactic acid fermentation is seen almost exclusively in cancer, in the absence of free hydrochloric acid. Gas production is notably observed in cases presenting a normal or increased hydrochloric acid content. The gases in question are carbon dioxide, oxygen, nitrogen, hydrogen, marsh gas, hydrogen sulphide, and in rare

instances, traces of olefiant gas. The oxygen and nitrogen represent air that has been swallowed. In an instance reported by Ewald and Ruppstein, in which alcohol, acetic acid, butyric acid, and lactic acid were found in the vomited material, and in which all the gases just mentioned could be demonstrated, the patient was occasionally able to light the eructated gas at the end of a cigar holder, where it burned with a faintly luminous flame. A similar case has been reported by McNaught. The presence of ammonia and hydrogen sulphide, of course, indicates albuminous putrefaction. Boas states that the sulphide is quite commonly present in cases of dilatation referable to benign causes, while it is almost always absent in cancer.

Microscopic examination reveals the presence of the *Boas-Oppler bacillus* with abundant lactic acid production in cases of cancer, while in non-malignant cases yeast cells and sarcinæ are frequently abundant.

The Urine.—The amount of urine is usually much reduced in advanced cases of dilatation, owing to deficient absorption of water; 500 to 800 c.c. are commonly found and at times the oliguria is still more marked. The specific gravity is correspondingly increased and on standing deposits of phosphates, notably triple phosphates, frequently separate out. The reaction is then usually alkaline. This is especially common in cases showing an abundant secretion of hydrochloric acid. In cases of anacidity, on the other hand, the reaction is acid. The chlorides are usually diminished. In some cases acetone, diacetic acid, and small amounts of albumin may be found. The former are especially apt to be met with in cancer of the stomach, and it is thought that they may be formed directly from the ingested albumins. This view is supported by the observation that in cancer acetone may be observed at a time when marked loss of flesh has not yet occurred, and that larger amounts may be found in the stomach than in the urine.

SYPHILIS

Essential Factors.—Secondary anemia; positive reaction with Justus' blood test; lymphocytosis; positive Wassermann reaction; demonstration of the Spirochæte pallida; positive butyric acid reaction (Noguchi) in the cerebrospinal fluid; positive Wassermann reaction in the cerebrospinal fluid; cerebrospinal lymphocytosis.

The Blood.—*The Red Cells and Hemoglobin.*—A certain degree of anemia of the secondary type is noted in all cases of active syphilis, its intensity varying with the intensity of the infection. The lowest red cell and hemoglobin values are reached just before or coincidently with the appearance of the rash. In the secondary stage the degree of anemia, *cæteris paribus,* may be regarded as a fair index of the severity of the infection. In untreated cases the hemoglobin remains

low for several days or even for weeks. A gradual rise then occurs which is associated with beginning involution of the exanthem. In uncomplicated cases normal values may subsequently be reached even without treatment. A fall occurs again when relapses develop. Similar changes are observed in the tertiary stage. In congenital syphilis, the anemia is usually marked, excepting in very mild cases, and in the severer infections the blood picture may simulate that of pernicious anemia.

Of interest are the observations of Justus on the blood changes which occur in the course of mercurial treatment. Justus ascertained that a rapid and material diminution of the hemoglobin (10 to 20 per cent.) occurs when a large amount of mercury is introduced at one time into the body of the infected individual (at least 3 grams of blue ointment should be used for an adult, and 1 gram for a child). This decrease is only observed in the blood of patients with florid syphilis; it is specific and does not occur in healthy or otherwise diseased individuals. The drop is obtained by the next morning, and is demonstrable in from 70 to 80 per cent. of active cases of all types of the disease, as soon as the more distant lymph glands begin to swell. It disappears, or is at least no longer demonstrable with beginning involution of the symptoms, and returns with the occurrence of relapses.

Various writers have confirmed the findings of Justus, and in special cases his *syphilitic blood test* may be useful as a diagnostic aid. The complement fixation method, however, gives more definite and satisfactory results, and will probably always be preferred (see below).

The Leukocytes.—With the appearance of the secondary symptoms there is usually a moderate increase of the leukocytes, which rarely exceeds 15,000 to 18,000, however; it is essentially referable to an invasion of lymphocytes, while the neutrophiles are, relatively at least, diminished. The lymphocytosis may persist for a long time; its duration depends upon the activity with which treatment is carried out. The highest grades are seen in children with congenital syphilis. The eosinophiles are not increased.

Da Costa mentions that he has found *iodophilia* in severe syphilitic anemia. In such cases there may also be a marked increase of the *plaques.*

Complement Fixation (Wassermann reaction).—While the search for the spirochete should be attempted in all primary lesions, the complement fixation method should subsequently be employed in the diagnosis of all suspected cases. The results are very satisfactory. The method is too complicated, to be sure, for common use, but the specimens can readily be taken at a distance from clinical laboratories and sent through the mail. If an interval of only forty-eight hours is to elapse between the taking of the blood and its arrival at the laboratory, the serum need not be separated from the corpuscles; otherwise, it is best to centrifugalize the specimen first and to pipette

off the serum. This will then keep for days, or even weeks, if pains have been taken to work aseptically. For a short transportation, asepsis is not necessary.

Examinations of this order have now been made in thousands of cases, and it may be regarded as an established fact that the reaction can be obtained in the blood or the cerebrospinal fluid in practically every case of syphilis in which the disease has not been eradicated by energetic treatment. It is to be noted, however, that a certain length of time must elapse after infection before antibodies can appear in the serum in appreciable amount, and that early cases of the disease hence furnish the lowest percentage of positive findings. As vigorous mercurial treatment, furthermore, may cause a temporary disappearance of the reaction, and as this has probably been overlooked in many cases, the percentages of some authors are lower than those of others. I append the findings of Noguchi which were obtained by him with his own system in 1082 cases of syphilis in its various phases and stages, and in a comparative study of syphilis and parasyphilitic conditions in 244 cases analyzed by his own and the regular Wassermann system, as also Kaplan's series of 1286 cases, in which the result of the two systems are contrasted. In suspected cases of syphilis of the central nervous system, in which a negative reaction is obtained with the blood, a corresponding examination of the cerebrospinal fluid should never be omitted. (See also p. 793.)

In alcoholic patients a negative reaction may be temporarily obtained, while after withdrawal of the stimulant the true state of affairs will be apparent.

Abderhalden Reaction.—In cerebrospinal syphilis (paresis) Wegener found that the serum gave a positive reaction only with brain tissue, while others have obtained an occasional reaction with testicle (in males) and liver.

NOGUCHI SYSTEM. SYPHILIS, PARASYPHILIS, HEREDITARY SYPHILIS, AND SYPHILIS SUSPECTS

	Cases examined.	+		—	±
		No.	Per cent.		
Primary syphilis	70	65	92.8	4	1
Secondary syphilis	197	190	96.0	5	2
Tertiary syphilis	177	159	89.9	16	2
Early latent syphilis	115	87	75.6	24	4
Late latent syphilis	150	119	79.3	27	4
Under prolonged treatment	39	4	10.2	32	3
Cerebral syphilis	5	3	60.0	1	1
Tabes	125	85	68.0	27	13
General paralysis	15	13	86.6	2	0
Hereditary syphilis	17	17	100.0	0	0
Syphilis (?)	172	60	34.8	96	16
	1082	802		234	46

Comparison of the Wassermann and Noguchi Systems

	Cases examined.	Wassermann			Noguchi		
		+		−	+		−
		No.	Per ct.		No.	Per ct.	
Primary syphilis	23	17	73.9	6	20	86.9	3
Secondary syphilis	79	69	87.3	10	76	96.2	3
Tertiary syphilis	65	52	80.0	13	57	87.6	8
Early latent syphilis	27	13	48.0	14	18	66.6	9
Late latent syphilis	32	24	75.0	8	27	84.3	5
Tabes	18	8	44.0	10	13	72.2	5
	244	183		61	211		33

Comparison of the Wassermann and Noguchi Systems (Results Obtained by D. M. Kaplan)

	Cases examined.	Wassermann			Noguchi		
		+		−	+		−
		No.	Per ct.		No.	Per ct.	
Primary syphilis	138	122	90	16	134	97	4
Secondary syphilis	281	242	86	39	270	98	11
Tertiary syphilis	191	140	73	51	155	81	36
Latent syphilis	79	41	51	38	60	75	19
Hereditary syphilis	20	18	90	2	18	90	2
Tabes	205	125	60	80	134	65	71
General paresis	61	40	65	21	44	72	17
Cases for diagnosis	311	98	31	213	180	57	131
	1286	826		460	995		291

Pregnancy apparently has an inhibitory effect upon the Wassermann reaction, such that in some instances it is negative while in others it is partial only. In this connection it is interesting to note that syphilitic women giving a positive reaction may give birth to children having no sign of syphilis but giving a complete or partial Wassermann. Then, again, syphilitic mothers with a partial reaction may have children with a more pronounced reaction than their own, while syphilitic mothers with a negative reaction may have children with a positive reaction. In doubtful cases it is hence advisable to view even a plus or plus over minus result with suspicion, if the woman is pregnant at the time of the examination, and to invariably examine the blood of the children.

Demonstration of the Spirochæte Pallida.—The demonstration of the Spirochæte pallida in suspicious lesions may be viewed as incontestable proof of the existence of syphilis. As this is practically only possible in the primary and secondary stages of the disease, the search for the organisms should only be attempted in such cases. In the primary lesions, the organism can probably always be demonstrated if the necessary material is procured under proper precautions (see

technical part). Sometimes they are quite numerous, while at others much patience will be required in the search. In secondary lesions, most writers report that they can also be found constantly; but the search is often very tedious owing to the character of the material.

Some writers claim to have found the spirochete in the blood (in untreated cases, during the secondary stage), but its search is practically a hopeless undertaking.

The Cerebrospinal Fluid.[1]—Cytological examination of the cerebrospinal fluid in syphilitic lesions of the central nervous system (general paresis, tabes, cerebrospinal syphilis, syphilitic hemiplegia) reveals a marked lymphocytosis in most cases. This observation is of importance in the differential diagnosis of a syphilitic meningitis or the early stages of tabes and general paresis, from other malignant processes and neurotic conditions. In tuberculosis, unfortunately, the same is found. Sometimes the increase is intermittent and paroxysmal. Noguchi has made a very interesting comparative study of his butyric acid test, the Wassermann reaction, and the cytological formula, *all three* applied to the *cerebrospinal fluid.* In the secondary and tertiary stages of syphilis without direct involvement of the nervous system the cerebrospinal fluid yielded a reaction of feeble intensity to the butyric acid test, while the Wassermann reaction (in this fluid) and the cytological formula were negative. The cerebrospinal fluid of a group of cases of hereditary syphilis gave a positive butyric acid reaction in about 90 per cent. and a positive Wassermann reaction in about 80 per cent. of those examined. On the other hand, the cerebrospinal fluid obtained from cases of cerebral and spinal syphilis yielded the butyric acid reaction in all cases, and at the same time gave a positive cytodiagnosis, while the Wassermann reaction was positive only in 50 per cent. of the cases. In general paresis the butyric acid test was positive in 90 per cent., cytodiagnosis in 91 per cent., and the Wassermann reaction in 73 per cent. of the cases examined. In tabes the corresponding figures were 100, 100, and 52 per cent. The butyric acid curve and the cytological formula thus run a parallel course in parasyphilitic disease, while the Wassermann reaction, as applied to the cerebrospinal fluid, is negative in a fairly large percentage of cases. In tubercular disease of the meninges the butyric acid test and the cytological formula would not be applicable for differential diagnostic purposes, as the findings are the same as in syphilitic disease; in such cases a careful search should be made for the tubercle bacillus and the Wassermann reaction applied both to the cerebrospinal fluid and the blood serum. In other diseases of the meninges, in which a positive butyric acid reaction might be obtained (acute inflammatory diseases), the cytological formula would, of course, lead to a correct diagnosis.

[1] As regards the colloidal gold reaction in syphilis affecting the central nervous system, see section on Examination of the Cerebrospinal Fluid.

TETANUS

Essential Factors.—Hyperleukocytosis with normal eosinophile values; occasional presence of the tetanus bacillus in the pus; irregular albuminuria and glucosuria.

The Blood.—Very little is known regarding the blood picture of tetanus. Cabot records the findings in three cases only. The hemoglobin was 70 per cent. in one and 80 per cent. in another. The leukocytes numbered 11,100 to 11,900 in the first, 19,600 in the second, and 18,200 in the third. All three were fatal, and had been treated with antitoxin. The writer remarks that the eosinophiles were not diminished.

Regarding the bacteriological findings also there is much uncertainty. Some investigators claim to have found the corresponding bacillus, while others obtained negative results. Considering the general properties of the organism, the latter would seem the more probable.

The Pus.—In the pus obtained from the wound through which infection has taken place, the tetanus bacillus can sometimes be demonstrated. How often this is possible is not known. Not infrequently the wound is so trivial that it is overlooked, which fact explains the occurrence of cases of so-called idiopathic tetanus.

The Urine.—In some cases small amounts of albumin and of sugar have been found. Strümpel mentions a case in which *sub finem vitæ* a croupous pneumonia and acute nephritis developed. The content of urea, kreatin, and kreatinin is not affected, while the amount of lactic acid is said to be increased.

THORACENTESIS

(Albuminous expectoration)

When a large accumulation of fluid is rapidly withdrawn from the pleural cavity it may happen either during the operation or within an hour following that the patient more or less abruptly begins to expectorate serous fluid in large quantities. The cause of this occurrence, which is fortunately rare, is unknown. In severe cases the onset is sudden, the fluid pours from the patient's mouth, and death may occur from suffocation; sometimes, indeed, the patient dies before the expectoration has properly begun. In milder cases no untoward symptoms develop, and the attack is over in a few hours. The amount of fluid which is brought up usually varies between 200 and 900 c.c., but may exceed a liter or even a liter and a half. On standing, trisedimentation occurs to a certain extent—a frothy white layer on top, a serous opalescent layer in the middle, and a somewhat more viscid turbid layer at the bottom, in which a few flocculi of fibrin and a small amount of blood may be distinguished;

in other cases only two layers can be made out—the main bulk being serous, with a thin frothy white layer on top. Chemical examination shows that the fluid is strongly albuminous and may resemble the corresponding pleural effusion in composition; this, however, is variable, and in some cases there is a marked difference between the two. Microscopic examination merely shows the presence of a few epithelial cells, leukocytes, and red corpuscles. The specific gravity in Riesmann's case was 1.018, with 5.84 per cent. of solids.

TONSILLITIS

Essential Factors.—Hyperleukocytosis and septic factor; bacteriological examination of the exudate usually shows predominance of pus organisms; tendency to albuminuria.

The Blood.—*The Red Cells and Hemoglobin.*—The red cells and hemoglobin are usually but little affected, but in the severer cases a moderate grade of anemia (of the secondary type) may develop quite rapidly.

The Leukocytes.—In mild cases the number of the leukocytes is not increased; in those of average severity there is a well-marked hyperleukocytosis, and in some instances the number may be quite high. In a series of 89 cases which I have collected from different sources, the count was 10,000 or more in 70, ranging between 10,000 and 41,000; values higher than 20,000 were found in 12 cases. The hyperleukocytosis is referable exclusively to an increase of the neutrophilic elements, while the eosinophiles show a distinct decrease; in other words, there is present the septic factor. The degree is usually moderate, the neutrophiles not exceeding 80 per cent., but in some instances it is quite intense. This is especially the case when abscess formation takes place. With the establishment of convalescence there is a rapid return to normal conditions, and as in other bacterial infections, in which hypoeosinophilia occurs, the return of the eosinophiles to normal values may be viewed as a favorable symptom.

The Bacteriological Blood Examination.—The bacteriological blood examination is usually negative, but in severe cases the corresponding organism may at times be isolated.

The Exudate.—Bacteriological examination of the exudate usually shows a predominance of the common pus organisms, viz., staphylococci or streptococci, but in some instances the pneumococcus, the Micrococcus catarrhalis, the Micrococcus tetragenus, the bacillus of sputum septicemia, and still others may be encountered as the principal offending bacteria. The flora is, of course, quite mixed. Meyer, in v. Leyden's clinic, succeeded in cultivating a diplostreptococcus from the tonsils in several cases of acute rheumatism with angina, which he views as the causative agent of the arthritic symp-

toms. Allaria reports similar findings. I have also observed a case of this kind in which a generalized infection (endocarditis, pericarditis, pneumonia, arthritis) developed, from which the patient died.

The Urine.—The urine usually presents those general characteristics which are common to all febrile conditions. Albuminuria and cylindruria of moderate grade are, however, not uncommon. Both generally disappear during convalescence, but exceptionally a tonsillitis may constitute the beginning of renal disturbance of longer duration. Sugar is absent, but a digestive glucosuria can in some cases be produced artificially.

TRICHINOSIS

Essential Factors.—Hypereosinophilia; lymphocytosis; presence of the trichinella embryos in the blood and muscle tissue.

The Blood.—*The Red Cells and Hemoglobin.*—In the majority of cases trichinosis does not produce any material decrease in either the number of the red cells or the amount of hemoglobin. Exceptionally a moderate grade of anemia of the secondary type is observed; this is more apt to occur in severe cases.

In my own person I found the *plaques* very much increased at a time when the eosinophile values were well up, and Schleip remarks that during convalescence the blood was actually swarming with them. I have only exceptionally seen them more numerous in other pathological conditions than in my own case. As Schleip found no evidence of degeneration of the red cells, he concludes that they were derived from disintegrating eosinophiles. My own impression led me to a contrary conclusion, without assuming the existence of an increased degeneration of red cells within the body.

The Leukocytes.—Through the investigations of numerous observers it has been established that trichinosis is almost invariably associated with a high grade of hypereosinophilia. There are few diseases, in fact, in which the increase is so extensive (as high as 86 per cent.), and in concrete cases these few can be readily eliminated by adequate examination. From the recorded facts it appears that in the diagnosis of trichinosis (in the absence of infection with other metazoic parasites) the results of the blood examination furnish more valuable information than the examination of bits of muscle tissue. Often it is possible to make a diagnosis of the disease from the high grade of the eosinophilia, where the clinical symptoms are indefinite or so insignificant as not to excite attention. The eosinophilia appears quite early. Schleip has shown that it may exist already at a time when the young parasites have not yet reached the muscle tissue. Whether it is demonstrable already within the first few days following infection has not yet been ascertained, however. The earliest date,

following infection in man, at which a satisfactory blood examination has been recorded, is the tenth (in one of Schleip's cases), with 44.5 per cent. of eosinophiles (7120 absolute eosinophile count). Generally speaking, the degree of eosinophilia runs a course parallel to the intensity of the infection and the number of the parasites. In very severe infections, however, the eosinophilia may fail to develop. Cases of this kind have been recorded by Da Costa, Howard, Dastre, Cutler, and Schleip. The same is seen in artificial infections in animals.

The grade of the eosinophilia which is observed in a concrete case, aside from the intensity of the infection, will depend upon the stage of the disease. The highest values (50 to 86 per cent.) are usually observed when the patients first come under observation. The number then gradually falls, but does not reach the normal line for a long time. In one of Brown's cases 16.8 per cent. was counted sixty-nine days after the patient was first seen. I, myself, was accidentally infected a few years ago and followed my eosinophile curve for three months. The initial figure was 50.7 and the last 7.3; six weeks later, *i. e.*, nineteen weeks after the first count normal values were again obtained.

The total number of the leukocytes is not materially increased in the mild cases, but in the severe infections there may be a very decided hyperleukocytosis. In Brown's first four cases the counts were 35,000, 13,000, 17,000, and 18,000.

Corresponding to the increase of the eosinophiles, there is a relative as well as an absolute decrease of the neutrophiles, with, at times, a marked diminution in the content of granules.

The lymphocytes show a gradual increase as the disease progresses, the maximal figures being reached by the time that the eosinophiles return to normal; after that the lymphocytosis may still persist for many weeks or even months.

Presence of Trichinella Embryos in the Blood.—During the stage of muscle invasion, the trichinella may be demonstrated directly in the blood, if a sufficiently large amount is examined. This should be attempted in all doubtful cases in which a marked hypereosinophilia is observed.

Demonstration of the Trichinella Embryos in the Muscle Tissue.—After the stage of muscle invasion has been passed, the embryos can be demonstrated *in situ* in practically all cases. This should be attempted in all doubtful cases. In the majority of cases in which an eosinophilia of high grade exists, and in which other conditions which lead to marked eosinophilia can be eliminated, there is scarcely any need of a search for the trichina in the muscles. I have gained the distinct impression that in such cases the high eosinophilia should carry greater weight in the differential diagnosis of the disease as a positive factor, than a negative result so far as a muscle examina-

tion is concerned. In such cases, indeed, I regard the latter as superfluous. If the patient, however, is seen for the first time when the eosinophilia is on the decline, or if the question comes up at a time when the eosinophile values are normal, then a definite diagnosis will not be possible, unless a direct examination of the muscle tissue is made. To procure the necessary tissue, a special "muscle harpoon" has been devised, with which bits of muscle can be torn away without the necessity of making an incision with the knife. Sometimes it is possible to obtain satisfactory results in this manner, but, on the whole, it is better to secure larger pieces for examination by direct incision. With the smaller bits, only a positive result is of value; a negative finding does not exclude the possibility that parasites may have been present in other portions.

Diarrhea and Vomiting.—Diarrhea and vomiting, particularly the former, are common symptoms at the outset of the disease.

The Urine.—The urine shows no deviations from the normal in cases of average severity.

TRYPANOSOMIASIS (SLEEPING SICKNESS)

Essential Factors.—Secondary anemia; normal leukocyte count; large mononucleosis; presence of trypanosomes in the peripheral blood and, in the late stages, in the cerebrospinal fluid.

The Blood.—*The Red Cells and Hemoglobin.*—From the relatively meager data upon the subject, it appears that anemia of moderate degree is practically a constant symptom of the disease. Usually the loss of red cells and hemoglobin amounts to about 20 to 25 per cent. of the normal. Values lower than 3,000,000, on the one hand, and 40 per cent., on the other are exceptional.

Morphological examination shows but little abnormality, excepting the occurrence of an occasional normoblast in the more markedly anemic cases.

The Leukocytes.—The leukocytes are not absolutely increased, but in many cases there is a relative increase of the large mononuclear elements (12 to 18 per cent.), as in malaria. The eosinophiles are not increased.

The Presence of Trypanosomes.—The diagnosis of the disease, in the early stages especially, should be based upon the demonstration of the corresponding parasite in the blood. This is, at times, exceedingly difficult, as the organisms may be present in very small numbers. As a general rule, not more than three to eight are found to a coverslip. Exceptionally they are numerous—up to 70 per smear. During apyrexial periods they are not seen at all in the peripheral blood. They may thus be absent for weeks and even months, and subsequently reappear. In doubtful cases it is well to examine the fluid obtained from the cervical lymph glands or from edematous areas,

in which they may be quite abundant. If negative results are obtained and the clinical picture nevertheless suggests trypanosome infection, a suitable animal (rat) should be injected with the patient's blood, using from 5 to 10 c.c. (See Parasitology of the Blood.)

The Cerebrospinal Fluid.—During that stage of the disease which has been described as "*sleeping sickness,*" trypanosomes can usually be demonstrated in the cerebrospinal fluid, obtained by lumbar puncture. Castellani obtained the organism in 20 cases out of 34, and Bruce found it in all of 38 cases. The results of these earlier observers have since been thoroughly confirmed. The organisms are most apt to be found toward the termination of the disease, while in the earlier stages of the infection they are absent. As in the blood, large numbers are rare, and their occurrence is usually associated with an access of temperature. When present, the leukocytes are apt to be increased.

There is no relation between the number present in the cerebrospinal fluid and in the blood.

The Urine.—Regarding the urinary picture there are no adequate data.

TUBERCULOSIS (ACUTE MILIARY)

Essential Factors.—Leukopenia; diazo reaction.

The Blood.—*The Red Cells and Hemoglobin.*—The red cells and hemoglobin are probably diminished in all cases after the disease has once become established, but owing to a concentration of the blood, which becomes the more marked the longer the disease has been in progress, the actual anemia is more or less obscured by a relative polycythemia and corresponding hemoglobin values. The color index, however, is commonly low. (See Pulmonary Tuberculosis.)

The Leukocytes.—The leukocytes are diminished in number in the majority of cases, excepting *sub finem vitæ,* when owing to complicating conditions hyperleukocytosis may occur. In a series of 17 cases studied at the Johns Hopkins Hospital, the leukocytes varied between 1000 and 9000, and 9 of these between 3000 and 6000. In Cabot's series of 36 cases, figures lower than 1000 were obtained in 25, and in only two of the entire number was the count higher than 10,000; in one instance it was only 550. Warthin mentions a case with 600. Complications may raise the count as high as 30,000, but minor events do not cause a very marked increase.

Differential counts in a large series are unfortunately not available. To judge from the very meager data which have been recorded, neutrophilic polynucleosis seems to be common. In Warthin's case the percentage was 91.4, and in a case mentioned by Simon and Spillmann it was 98. Future studies will have to show what variations one can expect. Emerson, commenting upon the cases observed at the Hopkins, states that one case had an interesting differential count,

viz., 81.9 of neutrophiles, but he does mention the findings in the others. According to Arneth, there is a marked anisohypocytosis with predominance of mononuclear forms.

The tubercle bacillus has been repeatedly found in the blood in acute tuberculosis, but the search is very tedious and often in vain. Nevertheless, a careful examination is indicated in doubtful cases; but only a positive result is of value. The antiformin method with an attempt at culture would suggest itself as particularly applicable.

The Sputum.—The sputum shows no characteristic features in acute miliary tuberculosis; it may, indeed, be absent; if present it is mucoid or mucopurulent and sometimes streaked with blood. Tubercle bacilli are commonly absent. (See also Acute Pulmonary Tuberculosis.)

The Urine.—The urine shows no characteristic changes. The color is usually a dark reddish amber. It is more frequently clear than that of typhoid fever, and also diminished to a greater extent; the density is high (1.028 to 1.032); the reaction is mostly very acid, but it may be alkaline; urate sediments are common. The chlorides are diminished, while the earthy phosphates are somewhat increased. The indican elimination is largely dependent upon the existence of intestinal complications; in their absence there is often no increase. The urohematin is always increased, and often very markedly so; hemaphein and uroerythrin may likewise be abundant. The urea is said to be increased. With the cold nitric acid test, the so-called uric acid ring may be so extensive that it may be confused with a large amount of albumin. The latter may be present in small quantity, but is not found with the same constancy as in typhoid fever.

The *diazo reaction* is, unfortunately, very common in acute tuberculosis, and may occur early in the disease. At this time, therefore, its presence is of no moment in the differential diagnosis from typhoid fever. But whereas the reaction disappears in typhoid fever in the third week, it may continue in tuberculosis to the fatal end. The recognition of this fact may at times be of service.

TUBERCULOSIS OF THE BONES

The Blood.—*The Leukocytes.*—In tuberculosis of the bones in contradistinction to osteomyelitis hyperleukocytosis is the exception and not the rule. When it occurs it is usually due to a mixed infection. In Fiske's series the count was below 12,000 in 66 per cent. of the cases.

TUBERCULOSIS (PULMONARY)

Essential Factors.—Secondary anemia with relative polycythemia; absence of hyperleukocytosis; lymphocytosis; demonstration of the tubercle bacillus (*a*) in the blood, (*b*) in the sputum; elastic tissue in the sputum; diazo reaction in the urine.

The Blood.—*The Red Cells and Hemoglobin.*—The blood findings in pulmonary tuberculosis depend upon the stage of the disease, the existence of fever, night sweats, diarrhea, hemorrhage, associated pyogenic infections, amyloid degeneration, etc.

Early in the disease, especially in fairly robust individuals, so long as the general nutrition is still good, the red count is quite commonly normal. But in patients who have repeatedly suffered from tubercular lesions since childhood, and in whom the onset of the pulmonary disease has been gradual, a marked loss in red cells is noted (4,000,000). In the secondary stage, owing to a concentration of the blood in consequence of a gradual dehydration of the body, normal figures are the rule; or if there be an oligocythemia, this is manifestly not in proportion to the manifest degree of anemia. This lack of proportion between the numerical findings and the pallor and emaciation of the individual is frequently very striking, and particularly so in the more chronic cases, in which septic symptoms are not marked. In the third stage oligocythemia is generally pronounced (2,000,000 to 2,500,000) and may be extreme (700,000). In rare instances the anemia may, indeed, assume the pernicious type; Ewing thus cites a case, reported by Hills, in which the red count dropped to 155,000.

The loss of hemoglobin is, generally speaking, proportionate to the loss of red cells, and in many cases exceeds the oligocythemia; in the third stage this is, in fact, the rule. The color index is thus either normal or somewhat diminished.

The morphology of the red cells shows no essential deviation from the normal, unless the anemia is extreme, when a certain grade of poikilocytosis may be observed. This, however, is rarely as marked as in other types of cachexia of the same degree. Granular degeneration plays no role, and even after hemorrhages it is uncommon to meet with nucleated red cells.

The Leukocytes.—Uncomplicated tuberculosis is not associated with hyperleukocytosis, and it is noteworthy that minor suppurative complications even do not cause a material increase in the number of the cells. With high septic fever, however, owing to recent cavity formation or an advancing pneumonic process, hyperleukocytosis probably always exists. It is, nevertheless, rarely as high as in the corresponding non-tubercular lesions. Ewing thus found both lungs consolidated and riddled with small cavities in a case which had lasted five weeks, where the leukocyte count had never exceeded 12,000.

Hemorrhages call forth a temporary increase, which depends in extent upon the amount of blood that has been lost (15,000). In the third stage, where pyogenic infections stand in the foreground, there is usually a leukocytosis of from 15,000 to 20,000.

When hyperleukocytosis does occur in the course of pulmonary tuberculosis, it is referable to an increase of the polynuclear neutro-

philes. But it is important to bear in mind that the tubercular process *per se* does not lead to a neutrophilic polynucleosis, but to a lymphocytosis. In non-complicated cases, therefore, the latter is the rule. How early this occurs has not been ascertained; in my own experience it is commonly well marked when the patients are first seen. The eosinophiles are usually found slightly increased, and it is noteworthy that even in those cases in which the neutrophiles are up the eosinophiles tend to persist. The septic factor, such as we see it in ordinary pyogenic infections, is thus frequently absent. The injection of tuberculin is said to give rise to eosinophilia in those cases in which a febrile reaction occurs. In one case reported by Grawitz it reached its highest value—41,000 absolute—three weeks after the injections had been stopped. Swan and Brösamlen report that an increase in the number of eosinophiles is noted within three to nine hours following the injection of tuberculin in tubercular patients, while this does not occur in non-tubercular individuals. The increase affects rather the total number than the percentage value of the eosinophiles.

On the basis of his study of 80 cases of lung tuberculosis Cummings concludes that the Arneth count has no diagnostic value, but assists in the prognosis, an increase in the forms with few nuclear lobes going hand in hand with a deterioration of the patient's condition and *vice versa*.

The Tubercle Bacillus in the Blood.—While some writers claim to have isolated the tubercle bacillus from the blood of tubercular patients, others have not been successful. Libman states that about twenty-four hours following the injection of tuberculin he obtained positive results in 56 out of 141 cases. Austrian and Hamman, on the other hand, state that they were unable to demonstrate the presence of tubercle bacilli in the blood in a single instance notwithstanding the fact that many of the patients were in the last stages of the disease. They do not believe that the bacilli are mobilized by the injection of tuberculin.

In advanced cases of phthisis various investigators have found pyogenic organisms in the blood, but it is possible that some of the results obtained were due to accidental contamination. Rosenberger states that he found associated organisms (pneumococci) in only one instance of his series.

The Sputum.—The amount and character of the sputum in pulmonary tuberculosis depends upon the stage and character of the disease. In acute cases there may be none at all. In acute pneumonic tuberculosis, for example, it is not infrequent to find no expectoration whatever for a number of weeks; when present in these cases it is usually at first like that of ordinary lobar pneumonia, viz., rusty and markedly tenacious. Subsequently it becomes thinner and green.

In the ordinary chronic type of pulmonary tuberculosis there may likewise be no sputum at first, even though physical examination may show the existence of a definite lesion. When present, it is frequently small in amount—only a little in the morning—purely mucoid and not at all suspicious looking. Nevertheless, it may even then contain tubercle bacilli in large numbers. Later, it becomes more and more mucopurulent, and finally almost entirely purulent. The sputa are then nummular in character, viz., when expectorated into water they sink to the bottom and there form greenish coin-like disks, from which property they have received their name. Such sputa are met with especially where active cavity formation is going on, but they are not characteristic and may also be derived from the larger bronchi. Quite different are the *sputa globosa*, which consist of fairly dense, roundish, grayish-white masses; these are secreted in old cavities which have become lined with a granulation membrane.

The amount of sputum which may be expectorated when the disease has become well established is quite considerable. With large vomicæ 100 to 150 c.c. may be coughed up in the morning, and in extreme cases the quantity may reach 500 c.c. or more.

Of late attention has been directed to the frequency with which *albumin* can be demonstrated in the sputa of tubercular patients, even at a time when bacilli are not yet present, and there is a tendency to lay some stress upon this factor in the diagnosis of early cases. I would suggest that the results of repeated examinations only be considered, and it should be borne in mind that a positive albumin reaction may at times be observed in benign affections of the lung and will naturally be obtained whenever blood is present in the sputum. It is interesting to note that the reaction in early cases of tuberculosis may be absent until a dose of tuberculin is administered and a focal reaction is thereby provoked.

Blood is present in almost all cases at some stage of the disease. Most frequently it is met with in incipient cases, and in many instances its occurrence is the first symptom which attracts the attention of the patient. In early cases the sputum is usually only streaked with blood, while larger hemorrhages are more apt to occur later. Unless it has remained for some time in a cavity, when it may be dark in color, the blood is bright red. Hemorrhagic sputa should always be examined for tubercle bacilli with great care, as they are apt to contain the organisms in especially large numbers.

Attention should also be directed to the presence of small cheesy particles—*corpora oryzoidea*—which are occasionally found at the bottom of the spit-cup. They vary in size from that of a millet-seed to that of a pea, and are observed especially in the second and third stage of the disease. They usually contain tubercle bacilli in large numbers and frequently also elastic tissue.

On microscopic examination, it will be seen that the cellular elements of the sputum are, for the most part, neutrophilic *leukocytes*. Some eosinophilic cells, however, may also be encountered. Teichmüller insists that the latter are present in large numbers, and may be found months before tubercle bacilli can be demonstrated. He regards their presence as evidence of a defensive struggle which is most evident in fairly robust individuals. With improvement, a gradual increase in their number is noticeable, while a diminution according to Teichmüller is indicative of a relapse, or, if the diminution occurs rapidly, of a florid process. These statements, however, lack confirmation and are unquestionably too dogmatic. Other observers, such as Ott, Fuchs, Bettmann, Turbon, and Cohn, deny that the presence or absence of eosinophilic cells in tubercular sputa is of any prognostic significance. Cohn, in fact, states that the occurrence of eosinophilic cells is fairly uncommon in tubercular sputa. Stadelmann also states that he has been unable to verify Teichmüller's statements.

Alveolar epithelial cells are present in all sputa of this order, but are of no special significance. *Blood corpuscles* will frequently be seen even where no blood can be detected with the naked eye.

The presence of *elastic tissue* in the sputum is evidence of the existence of a destructive lesion of the lung, and hence to be expected at some stage of the disease. In former years, before the discovery of the tubercle bacillus, much more importance was attached to its demonstration than at present, where the diagnosis of the disease is attempted at an earlier stage. Nevertheless, it is important to search for it, and it should be remembered that it is not infrequently met with in what otherwise appear to be very early cases. In other cases, as in caseous pneumonia, it may not be demonstrable.

The Tubercle Bacillus in the Sputum.—In the chronic ulcerative type of pulmonary tuberculosis tubercle bacilli can probably be demonstrated in every instance when sputum is available, if careful search be made. In acute tubercular bronchopneumonia also they may be present quite early. In the fibroid type of the disease, on the other hand, they may be absent during long periods, and in acute miliary tuberculosis they may not be demonstrable at all. In every suspected case frequent search should be made for their presence, and it should ever be remembered that a negative result is only of limited values.

The number of bacilli which may be found in a sputum varies greatly, and while, in general, it may be said that it is in direct ratio to the intensity of the disease, and may thus be considered of prognostic value, too much reliance should not be placed upon this statement, as in acute miliary tuberculosis, and in cases that have gone to the formation of cavities, the number may be small or they may be absent altogether. In an incipient case, on the other hand, in a little

mucoid sputum the number may be large. If the number of bacilli steadily decreases in a series of examinations at intervals sufficiently long, the patient may be regarded as improving, but here the constitutional symptoms and local signs give much more accurate information.

If on repeated examination, large numbers of tubercle bacilli are found, the disease has, in all probability advanced to cavitation (Brown).

In tabulating the number of tubercle bacilli in reports one may adapt Gaffky's scheme, modified by L. Brown, as follows ($\frac{1}{12}$ oil immersion; ocular 1; B. &. L.):

1. Only 1 to 4 in a whole preparation.
2. Only 1 bacillus on an average in many fields.
3. Only 1 bacillus on an average in each field.
4. 2 to 3 bacilli on an average to each field.
5. 4 to 6 bacilli on an average to each field.
6. 7 to 12 bacilli on an average to each field.
7. 13 to 25 bacilli on an average to each field.
8. About 50 bacilli on an average to each field.
9. 100 or more bacilli on an average to each field.
10. Enormous numbers on an average to each field.

An attempt has been made to attach prognostic significance to the form and grouping to the tubercle bacilli in the sputum. To judge from the experience gathered at Saranac, it appears that virulent and attenuated forms of tubercle bacilli possess practically the same morphology, and that short bacilli usually represent a younger growth. Arrangement of the bacilli in clumps is more apt to be found in the severer cases, but may occur in all (Brown).

Of the variations in number and form of the tubercle bacilli during treatment with Koch's tuberculin it is unnecessary to speak at this place, as the prognostic significance attaching to such variations is questionable.

The Feces.—Diarrhea is a common symptom in advanced cases of pulmonary tuberculosis, even though no intestinal lesions exist.

The Tubercle Bacillus in the Feces.—It has long been known that tubercle bacilli may be found in the feces where tubercular ulceration of the intestinal tract exists, but Rosenberger claims to have shown more recently that they may be found in practically all forms of tuberculosis, and that the organisms are in part eliminated through this channel, even in cases where no active symptoms exist. He suggests that feces be examined for tubercle bacilli as a part of the routine examination, and especially in suggestive cases, where no expectoration can be obtained. He states that in acute miliary tuberculosis the bacillus is always present in the feces. "The organisms, as a rule, are comparatively few in cases not plainly diagnosticated as tubercular but in well-marked cases of pulmonary or intestinal

tuberculosis they are comparatively abundant." The writer adds: "The finding of the tubercle bacillus in a spread is not always easy of accomplishment; it has frequently taken me at least one hour and sometimes as long as two hours to find three or four bacilli." Other investigators, unfortunately, have not been able to confirm Rosenberger's observations.

I would suggest that in all doubtful cases the examination be conducted with the antiformin method (which see).

The Urine.—The urinary examination shows no characteristic changes in pulmonary tuberculosis, unless we regard the frequent occurrence of the *diazo reaction* in this light. As a result of his investigations in this direction, Michaelis concludes that its presence indicates either that the process is very extensive or that it will progress very rapidly, and that the prognosis is grave. A cure, he believes, is impossible, and improvement, if any, only temporary. Clemens notes that of 100 cases of phthisis which ended fatally, 87 showed the diazo reaction; Rütimeyer obtained positive results in 85 cases out of 106 which died. Of 13 cases of acute tubercular pneumonia, Fränkel and Troje found a positive reaction in 11. Grundriss states that in his fatal cases the reaction was present without exception. Similar results have been obtained by Cnopf, Sée, Goldschmidt, and others. Michaelis himself reports that of 111 cases of phthisis which were admitted to the Berlin Charité with well-marked reaction, 80 died in the hospital, 13 were discharged unimproved, 3 were transferred to other hospitals, and 15 left unimproved. In other words, of these 111 cases, a fatal result was known to have occurred in 72 per cent. Stadelmann states that of 38 other cases with positive reaction 28 died in the hospital, *i. e.*, about 75 per cent. The subsequent fate of the remaining cases was not ascertained; but we may well assume that of these at least 50 per cent. died; so that we may formulate the general rule that a fatal result may be anticipated in about 85 per cent. of all cases of phthisis in which a positive reaction is obtained. Michaelis, moreover, suggests that the end may be expected to occur within six months from the time at which a persistent Ehrlich reaction is established. Exceptions occur, but the above is the rule. In Koch's institute at Berlin patients presenting the diazo reaction are not treated with tuberculin (Brieger).

Ehrlich's benzaldehyde (urobilinogen) reaction, in my experience, is likewise not uncommon in tuberculosis, though it is in no sense characteristic. I have found it more frequently in the actively progressive cases than in those which are more or less stationary, and accordingly quite commonly associated with the diazo reaction.

Advanced cases with much febrile disturbance show those urinary changes which are the outcome of that condition. A trace of *albumin* and a few hyaline *casts* are then not uncommon. *Acetonuria* of moderate grade may also be observed; so also *diaceturia*, although

this does not seem to depend upon the fever *per se*, but rather upon the diminished ingestion of food. *Albumosuria* has been noted when suppurative processes are especially active.

TUBERCULOSIS OF THE SEROUS MEMBRANES

(Pleurisy; peritonitis; pericarditis)

Essential Factors—Secondary anemia; relative polycythemia; tendency to normal leukocytosis; lymphocytosis; normal eosinophilia; high specific gravity of the exudate; lymphocytosis in the exudate; presence of the tubercle bacillus.

The Blood.—*The Red Cells and Hemoglobin.*—The red count and hemoglobin values are sooner or later diminished in all cases, the extent of the anemia depending essentially upon the extent of the tubercular process and its duration. (See Pulmonary Tuberculosis.) At the time when the patient first comes under observation there may be no anemia, however, discoverable by the usual methods, although at that time already a concentration of the blood, as a whole, may exist and obscure an actual anemia.

The Leukocytes.—Tuberculosis of the serous membranes *per se* does not lead to hyperleukocytosis; this develops, however, if a secondary infection takes place. But even then the increase of the leukocytes is usually not so great as in the corresponding infections of the serous membranes in the absence of a tubercular lesion. In Cabot's series of 60 cases of tubercular peritonitis, the leukocyte count did not exceed 10,000 in more than 14 cases, and it was lower than 7000 in 26. In his series of 314 cases of serous pleurisy, many, if not most, of which were tubercular in origin, counts lower than 10,000 were obtained in 210. Three cases of tubercular pericarditis which the same writer mentions likewise showed no hyperleukocytosis Differential counts in large series are, unfortunately, not available, but my own impression has been that in the absence of septic complications there is a distinct tendency to increased lymphocyte values. The eosinophiles are, at the same time, apt to be maximal in number.

Exudates.—The exudates in tuberculosis of the serous membranes vary greatly in quantity; sometimes only a small amount of fluid is obtained, at others several liters may be withdrawn. The fluid is usually fairly clear or but slightly turbid, straw-colored or pinkish, owing to the admixture of a little blood; on standing this gives rise to the formation of a coagulum which becomes the more extensive the more blood is present. The specific gravity usually exceeds 1.018 (1.012 to 1.024), corresponding to the presence of a large amount of albumin.

Cytological examination shows a marked preponderance of lymphocytes over granulocytes, a factor which is most important in the diagnosis of the tubercular type of the lesion. This is true especially

for the *pleural* effusions, where the existence of a granulocytosis, excepting in the very earliest stage of the disease, will almost always rule out a tubercular process. In the later stages of the disease especially the lymphocyte is certainly the predominating cell. As a rule, the percentage will be found to range between 50 and 98 per cent. In peritoneal effusions the cytological formula does not give indications which are quite as direct, since peritoneal carcinomatosis and syphilitic lesions of the abdominal viscera associated with effusions will similarly produce a lymphocytosis. Nevertheless, the count is important, and frequently plays a prominent role in the diagnosis of tubercular peritonitis. Exceptions, no doubt, occur, but they are not so frequent as to invalidate the importance of the examination.

The Tubercle Bacillus.—The search for the tubercle bacillus in tuberculosis of the serous membranes is notoriously unsatisfactory. The result is so uniformly negative that in former years this fact alone was viewed as presumptive evidence of the tubercular character of the lesion. It is now known, however, that *all* exudates gradually become free from bacteria, even though at first they may have been caused by bacterial action. Conclusions are hence no longer justifiable upon this basis. A few years ago, Jousset and Zebrowski advocated a certain technique (inoscopy) for the search of tubercle bacilli in exudates (which see), with which they claimed to have obtained much more satisfactory results than former investigators. Others, however, have not been so successful, and in doubtful cases the animal experiment offers the best outlook.

TUBERCULOSIS OF THE URINARY TRACT

(Renal tuberculosis)

Essential Factors.—Polyuria; hematuria; pyuria; acid urine; presence of the tubercle bacillus.

The Blood.—*The Red Cells and Hemoglobin.*—The effect of a tubercular lesion of the urinary tract upon the red count and hemoglobin value will depend upon the duration of the disease, its extent, the existence of associated infections, etc. The general effect is essentially the same as that produced by a tubercular process in the lungs (which see), but it is remarkable to note how little anemic some of the cases really are in whom the tubercular process has already advanced fairly far. When anemia exists it is usually of the chlorotic type.

The Leukocytes.—The leukocytes are not increased in early cases. Subsequently, when secondary infection or active resorption of broken-down tissue is taking place, a hyperleukocytosis of variable degree is noted. Often the figures even then are not as high as one would expect, which, no doubt, is due to the fact that much of

the waste material is carried off successfully through the urinary channels. Exacerbations in the counts may at times be due to temporary blocking of the ureters. The differential count will usually show the septic factor, if active symptoms exist, but at times the eosinophiles persist. This association of normal eosinophile values with a polynucleosis should always excite suspicion.

The Urine.—The urinary picture in renal tuberculosis will depend to a great extent upon the seat of the disease, more particularly upon the involvement or non-involvement of the pelvis. If this is normal the urine may be normal. It is thus quite possible to find at autopsy a kidney that is almost wholly destroyed with no urinary abnormality during the life of the patient. It should further be borne in mind that temporary blocking of the ureter of the affected side, or lack of secretion, may be responsible for the excretion of apparently normal urine in cases in which a previous examination has shown marked abnormalities.

Early in the disease *polyuria* amounting to one-half of the normal amount, with or without albuminuria, is of frequent occurrence. The first symptom, however, which commonly attracts the attention of the patient is the passage of *blood*. The amount is variable; sometimes the bleeding is microscopic, while at others almost pure blood is passed. It is usually intermittent, the period of bleeding lasting from one hour to several weeks, the average being three days. Late in the disease it is usually less in amount, but apt to be continuous. As a rule, the blood and urine are intimately mixed; clotting, however, may occur in the pelvis of the kidney, the ureter, or the bladder. Unlike what we see in hematuria due to calculus, the blood is present at all times, bearing no relation to the position of the body; it is hence found in the early morning specimen as well as in the one voided in the evening.

The Pus.—Pus is present in all cases in which the pelvis of the kidney is involved. It appears early, but the amount is extremely variable. Sometimes only a few leukocytes are seen, while at others the pus may amount to one-fourth or even one-half of the urine by volume. As a rule, the pyuria is constant, but cases are also seen where for months, or even years, the urine may be almost clear. This may be due to improvement in the local condition, but not infrequently it is referable to blocking of the ureter of the affected side, or to a temporary cessation in secretion.

The Reaction.—The reaction of the urine in tubercular pyuria is almost always acid.

Albumin is, of course, always demonstrable whenever blood or pus is present; the latter by itself is only responsible for a trace. *Casts* may be present, but are not a constant factor in the urinary picture.

While the association of hematuria and pyuria with an acid urine should always excite suspicion, the diagnosis of renal tuberculosis

demands the demonstration of *the tubercle bacillus* as well. This is probably always possible when the pelvis of the kidney has become involved, even quite early in the course of the disease; it may, however, be a very tedious matter. Attention should be especially directed to the presence of tiny bits of cheesy material, which are often extremely rich in bacilli. In some cases, where caseous material of this sort is present, thousands of bacilli may be found closely matted together. On the other hand, the number may be quite small. In some cases the animal experiment will be necessary to come to a satisfactory conclusion. The antiformin method may be used to advantage in these cases. *Elastic tissue* may also be encountered.

TYPHOID FEVER

Essential Factors.—Secondary anemia; hypoleukocytosis; absence of the septic factor; lymphocytosis and splenocytosis; absence of iodophilia; presence of the typhoid bacillus in the blood; Widal reaction; diazo reaction; presence of the typhoid bacillus in the urine; tendency to albuminuria and indicanuria.

The Blood.—*The Red Cells and Hemoglobin.*—In all cases of typhoid fever there is a loss of red corpuscles; but this is frequently obscured by a general concentration of the blood, so that normal or even supernormal values may be obtained during the active stage of the disease, viz., 4,000,000 to 7,000,000. During convalescence, however, the resultant anemia is quite apparent. Ewing found an average loss of from 100,000 to 500,000 cells per week during the first five weeks of the disease. Thayer gives the following average values: First week, 5,636,000; second week, 4,960,599; third week, 4,951,535; fourth week, 4,038,333; fifth week, 3,856,786; sixth week, 4,364,350.

While the anemia is thus usually quite moderate, cases do occur in which it is most intense. Henry has reported one instance, in which the red count had fallen to 804,000, and Thayer mentions two cases with 1,300,000 and 1,996,000 respectively. Anemia of this type may continue far into convalescence, and constitutes in itself a very important complication.

The loss in *hemoglobin* exceeds that of the red cells, so that, notwithstanding the concentration of the blood, the anemia is quite apparent. The average loss per week during the first five weeks is about 5 or 6 per cent. (Ewing). The color index varies between 0.7 and 0.8 (Thayer). In the severe cases also the loss of hemoglobin is, in a general way, proportionate to the loss of red cells, but the type of the anemia inclines toward the chlorotic. In one of the Hopkins cases, in which the red cells numbered 1,300,000, the hemoglobin had dropped to 20 per cent.

In cases of hemorrhage the resultant anemia corresponds to the

amount of blood which is lost and is comparatively rapidly compensated.

Qualitative changes in the red corpuscles are usually only seen in cases associated with marked anemia, but are not in any way characteristic. Nucleated red cells are, on the whole, rare; an occasional normoblast may, however, be encountered. They are more numerous in the hemorrhagic cases, and on rare occasions actual blood crises may occur. Megaloblasts are only exceptionally seen in the very severe cases with anemia, and more commonly in children than in adults.

The Leukocytes.—During the first few days of the fever there is sometimes a moderate hyperleukocytosis, but in most cases the number is not increased. The general tendency is toward a distinct leukopenia, which usually is quite manifest in the second week and continues until convalescence. This is so constant that we can formulate the general rule that whenever an increase of the leukocytes is observed in a case of supposed typhoid fever it is more than probable that some complication exists or that the diagnosis is wrong. Exceptions are rare. The extent of the leukopenia seems to depend to a certain degree upon the intensity of the infection and its duration. Favorable cases do not, as a rule, give counts which are materially below the minimal normal number, but in severe cases a drop to 2000 or even lower is not uncommon; in some instances the number may, indeed, fall to below 1000. Some observers have reported cases in which no leukopenia occurred and in which the number remained above 10,000 throughout the course of the disease without any complication. This is rare, and if unsupported by positive bacteriological findings I should be inclined to doubt the correctness of the diagnosis.

While a knowledge of the absolute leukocyte count is of material value in the diagnosis of typhoid fever, more important information is furnished by the differential count. During the first days, while the temperature is steadily rising, there is said to be a neutrophilic hyperleukocytosis of moderate degree, accompanied by a corresponding decrease of the mononuclear elements and disappearance of the eosinophiles. I have no personal counts of early cases, but Higley and Wood both were unable to demonstrate such an initial rise of the neutrophiles, and found instead a leukopenia with a relatively high lymphocyte count. In any event there is subsequently a diminution of the neutrophiles. The lymphocytic and the polynuclear curves usually cross about the end of the first week, so that a normal differential count is obtained at this time (the eosinophiles, however, being diminished or absent). This is very suggestive; so much so, in fact, that I always suspect typhoid fever, if such findings are recorded in a patient who has been ill with fever for a week. During the stage of continued fever the neutrophiles usually number from 50 to 60 per cent., while the mononuclear elements show a

proportionate increase with absence or subminimal normal values of the eosinophiles. During the third stage (remission) the neutrophiles may decrease still further (to 1500 to 2500 actual value), with a corresponding rise of the mononuclears; a few eosinophiles now also appear. In the fourth stage (defervescence) the neutrophiles reach their minimum—900 in severe cases—while the lymphocytes attain their maximal values (60 per cent., or more) and the eosinophiles gradually return to normal. The reascent of the neutrophiles then occurs very slowly, while the mononucleosis, most markedly so in children, continues far into convalescence. Normal values are sometimes not reached until after several months. After the eosinophiles have reappeared, there is early in convalescence a distinct tendency to a temporary hypereosinophilia (epicritic eosinophilia); this is only of brief duration, however, and may readily be overlooked.

While many writers speak indiscriminately of an increase of the lymphocytes in typhoid fever, I would emphasize that we are dealing essentially with a mononucleosis in which both the small lymphocytes and the large mononuclear elements (splenocytes) are increased. Ewing states that he found a uniform relation between the lymphocytosis in the blood and the grade of lymphatic hyperplasia as seen at autopsy. He records an instance in which the examination of the blood led to a strong suspicion of lymphatic leukemia and in which at autopsy the mesenteric glands were of unusually large size and the edge of the partly necrotic intestinal ulcers rose 1.5 cm. above the mucosa.

The Arneth count shows that the neutrophilic hypoleukocytosis is of the aniso type, with marked diminution of the polynuclear elements. Metamyelocytes, however, are usually not seen until convalescence begins, and then they occur only in small numbers. A few phlogocytes, especially in children, are frequently seen during the height of the disease.

Iodophilia is not commonly seen in typhoid fever before the end of the second week, and may be absent throughout the course of the disease.

In the event of a relapse occurring during an afebrile period there is, according to Nägeli, a distinct neutrophilic hyperleukocytosis, the actual number depending upon the preceding counts with the addition of from 3500 to 5000 neutrophiles; at the same time the eosinophiles disappear. This also happens if the relapse occurs in the third stage of the disease, when the cells had just begun to reappear.

Favorable indications in typhoid fever are: A return of the eosinophiles at the height of the disease and a steady increase to normal or supernormal values as the disease progresses; not too great a drop of the neutrophiles, with a corresponding increase of the mononuclear elements. Unfavorable indications are: Profound leukopenia, con-

tinued absence of eosinophiles, absence of hypoleukocytosis and a further decrease of the neutrophiles in the event of complications which *per se* would call forth an increase in the number of these cells.

When complications supervene in the course of typhoid fever the total count and the relative values will depend to some extent upon the nature of the offending organism. If this be the typhoid bacillus itself, there may not be a very material increase of the cells beyond normal and the differential count may show no marked qualitative changes. As a rule, however, there will be a hyperleukocytosis as compared with the findings just preceding the complicating condition. Conversely, infection with the typhoid bacillus should be suspected, if, in an inflammatory condition, which ordinarily would give rise to its appearance, the septic factor is absent or but little pronounced. I would emphasize particularly in such cases that stress be laid primarily upon the differential and secondarily only upon the absolute count. If, on the other hand, the complicating condition be referable to a superadded infection with one of the common pus organisms, the septic factor will be present, although its intensity may be somewhat diminished by the coincident typhoid infection. In cases of hemorrhage a moderate grade of hyperleukocytosis usually develops within twenty-four hours, and may persist for several days. In cases of perforation there is frequently an increase in the total number of the leukocytes to 10,000 or more, which may, however, be quite transitory and escape observation unless an early examination is made and previous counts are available; later, when peritonitis is general the leukocytes are usually diminished. In especially malignant instances the initial increase also may be lacking. In one of Cabot's cases the count before perforation was 8300 and immediately afterward 24,000. Finney reports a case with 6500 before and 10,600 after. In one of Cushing's cases there was an early recognized hyperleukocytosis which appeared before any sign of general peritonitis had developed—8400 before and 16,000 afterward; in this patient, the leukocytes fell to 4000 after operation, but immediately following the development of obstruction, due to kinking of the bowel, the number rose to 13,000 and later to 20,000, to fall again after the removal of the obstruction. In a second case there was a persisting hyperleukocytosis, associated with abdominal pain and tenderness, at one time reaching 15,200. Upon the development of general peritonitis the count dropped to 4300. In interpreting the leukocyte curve in suspected cases of perforation great caution is necessary. As Cabot remarks, "a steadily increasing leukocytosis is always a bad sign, and should never be disregarded, even when other bad symptoms are absent," but Cushing very appropriately adds, "A decreasing leukocytosis may be a much worse sign." *The differential count in cases of perforation shows the septic factor, no matter what the*

total count may be. In a recent case which fell under my observation the total count, three hours after the first symptoms, was 4000 and the neutrophilic count 85 per cent.

Bacteriological Examination of the Blood.—Since the introduction of ox gall as a medium for the cultivation of the typhoid bacillus the percentage of positive findings in the blood, early in the disease, has risen so markedly that this method can now be regarded as the most satisfactory in the early diagnosis of the disease. Taking the cases reported by Kayser, Veil, and Peabody, we find that of the 85 which were examined during the first week, a positive result was obtained in over 92 per cent.; of the 212 cases from the second week, in 66 per cent.; and of the 124 cases from the third and fourth weeks collectively, in 39 per cent. Coleman and Buxton further obtained positive results in all of 24 cases, in which the examination was made between the fifth and the twenty-first day of the disease. These results for the first week of the disease are so much better than those obtained with the agglutination test that the bacteriological examination should be resorted to in every case at this period of the malady. During the second week the values of the two methods is about on a par, while subsequently the agglutination test is the more important. If the first examination shows no organisms, further cultures should be made, the blood being taken from the ear, if for any reason vein puncture cannot be done.

The Widal Reaction.—While a positive Widal reaction may be obtained as early as the first day of the disease, meaning thereby the first day that the patient spends in bed, or the fifth of general malaise, such an occurrence must be viewed as a great rarity. In the vast majority of cases a positive result is obtained only after the fifth or sixth day in bed. As the likelihood of positive bacteriological findings is greatest during the first week of the disease, an examination in this direction may well take precedence over the agglutination test. During the second week, when the value of the two methods is on a par, convenience may decide which is to be employed. After this, however, the agglutination test should be given the preference. Experience has shown that a positive reaction may be obtained in practically all cases of true typhoid fever, but it is clear from what has been said that much depends upon the period of the disease at which the examination is made. The production of agglutinins evidently does not begin at the same time in all cases, and does not become fully established until after the disease has progressed for a certain length of time. It may happen, indeed, that a positive reaction is not obtained until convalescence, or even until a subsequent relapse occurs. For this reason it is advisable to repeat the examination at frequent intervals, if on first trial a negative result is obtained. Intermittence of the reaction, moreover, is quite common and emphasizes the necessity of frequent examinations still further.

While in some instances the reaction disappears very soon after the temperature has returned to normal, and even earlier, it generally continues well into convalescence, and may, in some instances, be obtained after months and even years following the attack. In a series of 71 post-typhoid cases, Krause found the reaction in 36, viz., in 16 of 26 cases examined within a year, in 12 of 21 examined between the second and the fifth year, in 7 of 19 between the fifth and the tenth, and in 1 case out of 5 between the tenth and twentieth (twelfth) year. Scott mentions a case in which he could demonstrate the typhoid bacillus in the feces twenty-two years following an attack of the disease and in which the Widal reaction was positive. In three instances no reaction could be obtained within a month of the disease. To what extent the continued presence of typhoid agglutinins may be referable to the persistence of the corresponding bacilli in the body has not been ascertained. It is known that they may persist in the gall-bladder and in the urinary bladder for a long time, and in several instances they have been found where no history of an antecedent typhoid fever could be obtained. In a case of cholelithiasis, reported by Cushing, typhoid bacilli were found in the gall-bladder, and distinct clumping obtained with a dilution of 1 to 30, although the individual gave no history of typhoid whatsoever. Cases are occasionally seen which clinically resemble typhoid fever very closely, but which do not give the Widal reaction at any time, with the usual dilution of 1 to 50. Some of these cases are referable to infection with organisms which are closely related to the typhoid bacillus and which also give rise to the formation of agglutinins. These, however, do not react with the typhoid bacillus excepting in low dilution. (See Paratyphoid Fever.) Infection with related organisms may also be responsible for certain cases of febrile jaundice, in which agglutination of the typhoid bacillus has been observed. In others the reaction may be due to a *localized* infection with typhoid bacilli. The biliary constituents in any event are not responsible for the reaction. This is clear from the observation of Kämmerer, who obtained agglutination in only 3 cases of jaundice out of 50, selected at random.

At present where antityphoid vaccination is carried on so extensively it is advisable to bear in mind that this will give rise to a positive Widal, and it is hence well to interrogate the patients in reference to this point. As regards the question how long agglutinins remain after inoculation with antityphoid vaccine Wollstein found that the agglutinin content reached its height within two months after the first inoculation or one month after the third injection and then fell rapidly within the next two months. 19 of 24 cases could be followed for a longer period. Of these 8 were negative after two months and 15 after thirteen months. One serum reacted yet in a solution of 1 to 1200 after that period of time.

The Feces.—The feces may not show any material deviation from the normal, considering the diet of the individual, but in many cases in which diarrhea is a factor the appearance is somewhat characteristic and has been likened to that of pea soup; this is especially apt to occur in the second and third weeks. The odor is very offensive and the reaction usually alkaline. The presence of traces of blood is not uncommon, and frequently precedes the occurrence of a notable hemorrhage. Pus is only seen in cases with very extensive ulceration. The microscopic examination shows nothing that is characteristic. Triple phosphate crystals, presenting the well-known coffin-lid form, are frequently seen and were at one time regarded as peculiar to typhoid fever, but they may also be encountered under normal, as well as under the most diverse pathological conditions.

The search for the typhoid bacillus is more complicated in the feces than in the blood, and not so likely to be of aid in the early diagnosis of the disease. Using special media (which see), a number of observers have reported favorable results, however, and it is possible that during the second week especially the method may at times prove of value.

The Urine.—The urine presents all those characteristics which, in a general way, are the outcome of the febrile process *per se*, but in addition there are certain deviations from the normal, which are more or less specific. The following rather detailed account of the general characteristics is taken from Robin:

The *color* is probably always more or less intensified during the active stage of the disease. During the period of ascending and stationary temperatures a brownish yellow is the rule, with more or less marked reddish or greenish reflexes. With the occurrence of defervescence the greenish reflex disappears and the urine becomes distinctly orange. With approaching convalescence it turns a pale yellow and is sometimes almost colorless. These variations are seen more especially in cases of moderate severity. In the severer types there is a more marked tendency to dark brownish and reddish tints at the height of the disease, while the orange tones persist for a longer time and may even be observed well into convalescence. In fatal cases the urine not infrequently presents a bouillon color throughout, in which a bluish green predominates and tends to obscure any reddish tint that may be present. In others the bluish green rapidly changes to a reddish ochre, then to a yellowish red, and ultimately to a yellowish brown with a distinct greenish reflex. In the renal type of the disease the urine shows the characteristic color which is seen in acute parenchymatous nephritis; it becomes a blood red, which masks the greenish tint almost entirely, as seen in the chamber.

The urine is usually more or less turbid when seen in the laboratory, though it may have been clear when passed. *Unless preserved artifi-*

cially, it rapidly undergoes decomposition. This tendency is very marked in typhoid fever. On standing in a cool room *urate deposits* commonly develop.

The Reaction.—During the first two periods the reaction is very acid. It then diminishes very markedly and becomes alkaline either during the period of defervescence or in the course of convalescence. The period of alkalinity may only exist for twenty-four hours; or it may continue for five or six days. The alkalinity is due to fixed alkali.

The Odor.—According to Robin the odor is more aromatic in the beginning, ammoniacal or even fetid during defervescence, while during convalescence it again becomes normal. During early convalescence it is often insipid, and this is often noticeable from the very beginning in fatal cases. In some cases an odor of hydrogen sulphide develops after defervescence and during that period.

The Specific Gravity.—The specific gravity depends essentially upon the amount of liquid ingested, the amount secreted, the amount eliminated through other channels (diarrhea, sweating), etc. It is usually highest during the first week, a little lower in the second, still lower in the third and fourth weeks, declining in a general way as the amount of urine increases.

Hewetson gives the following figures (all the cases were bathed):

First week	1.024
Second week	1.022
Third week	1.018
Fourth week	1.019
Fifth week	1.014
Sixth week	1.016
Seventh week	1.013

The Amount.—During the period of ascending temperature and the fastigium the amount is somewhat diminished, while the density is comparatively increased. During the period of descending temperature the amount increases and approaches the normal; at the same time the specific gravity falls a little, but still remains above the normal. With convalescence the amount increases beyond the average normal, while the specific gravity falls. This, at least, seems to be the rule in cases of moderate severity.

In the mildest cases there is but little deviation from the normal; if anything, the density is a little increased (1021.3). The *polyuria of defervescence and convalescence* is especially marked in the long-continued and grave cases (1685 c.c.). In fatal cases the quantity is diminished (922), while the specific gravity (1021.6) is not correspondingly increased. The diminished amount, lowered specific gravity and lowered solids in the fatal cases, begins early and continues to the fatal end in the renal and adynamic forms, while

in the thoracic form the amount is increased during the last day, coinciding with breaks in the diarrhea.

First and second periods.	Third period.	Convalescence.
1038 c.c.	1213 c.c.	1491
1.024	1.0199	1.0178
52.30 solids	53.40 solids	56.29 solids

The Mineral Constituents.—The mineral constituents are much diminished during the fastigium of the disease. With the occurrence of defervescence they increase, and reach normal values again at the time of convalescence. The diminution in the amount of mineral solids is largely at the expense of the *chlorides*. These are manifestly retained in the body, as Parkes could show that their amount is markedly diminished even though the patient be on a fairly general diet and has neither pneumonia nor diarrhea. A drop beneath 2 grams, however, rarely occurs in cases which recover. Robin gives 3.70 grams as average during the fastigium, 7.20 for the period of defervescence, and 14 for convalescence. In fatal cases the average is somewhat lower, viz., 2.5. The curve of the chlorides is thus of some diagnostic and prognostic value. The imminence or occurrence of defervescence is indicated by a crossing of the curve of the chlorides and that of the extractives.

What has been said of the chlorides holds good in a general way also for the *phosphates*. The diminution in the beginning principally affects the earthy phosphates. In fatal cases an increased elimination is sometimes observed.

The *sulphates* are somewhat increased in the early stages of the disease, while during convalescence and defervescence they tend to diminish below the normal.

The elimination of *urea* is quite variable. This is, of course, what one would expect, bearing in mind individual peculiarities of nutrition, irregular retention and elimination, the possibility of loss through other channels, the intensity of the toxemia in its effect upon the body tissues, the character of the diet, the amount of food ingested, etc. The average figures obtained by Robin for the different stages of the disease vary between 22.1 and 23.7 grams in the severe cases, and between 16.35 and 25 in those of moderate intensity. No relation exists between the height of the fever and the amount of urea, and in fatal cases the smallest amounts are eliminated, even though the temperature may be especially high. Generally speaking, the elimination of urea is lower when the typhoid symptoms are most pronounced, while the highest values are found in those cases in which the disease follows a frank, inflammatory course.

Generally speaking, the amount of *uric acid* is not as much increased in typhoid fever as in the frank inflammatory diseases. The largest quantity is found during the period of ascending temperature, when it sometimes amounts to 1.2 or 1.6 grams. In fatal cases there

is usually an increase; but this disappears with the approach of death in the renal and adynamic forms, while in the algid and asphyctic types it persists. A complicating pneumonia, intestinal hemorrhage, and pericarditis produce a more or less marked increase (Robin).

Albumin.—Regarding the frequency with which albumin occurs in typhoid fever, opinions differ. Gubler thought that it is present at some time in the course of the disease in all cases. Robin arrived at similar conclusions. In Hewetson's series of 229 cases, albuminuria was noted in 164. The albuminuria begins early in the disease and may be demonstrable on the second day. Its subsequent course is variable; the highest elimination in non-complicated cases occurs about the end of the first week, after which it diminishes and may disappear for one or two days about the end of the fastigium. In the grave cases the albuminuria undergoes a recrudescence during the greater part of the descending oscillations, and often even as early as the last two days of the fastigium. In the cases of moderate severity the increase during defervescence is limited to the beginning of this period, after which the amount falls to traces; it may, indeed, disappear, but it is rare that this second disappearance is final. In the grave cases the albuminuria persists, and it is unusual to see it disappear before the tenth day of convalescence; generally there are only traces, but at times more notable amounts are seen. In the more moderate cases the albumin frequently reappears during early convalescence in traces, but disappears finally after the first week. A relapse is commonly preceded by an increased degree of albuminuria. When the patient first turns to a more general diet, traces of albumin are frequently observed. Large amounts of albumin may be seen if actual nephritis accompanies the disease.

Glucosuria.—Glucosuria is not a feature of typhoid fever; the digestive form is, however, occasionally observed.

Indicanuria.—Increased indicanuria is a very common event, and especially marked in the severer forms of the disease. It is most intense at the height of the malady, and then steadily diminishes with defervescence and the establishment of convalescence. The highest grades are seen in those cases in which diarrhea is marked, or in which peritonitis develops.

The Diazo Reaction.—The diazo reaction is one of the most constant symptoms of the disease, but may only be present for a short while, and hence be missed in hospital cases more particularly, where the patients are not usually seen from the start. For this reason many of the statistics do not furnish a fair index of the frequency of the reaction. All writers who have carefully studied the question agree that it is rarely absent, and regard it as a valuable symptom, notwithstanding the fact that it is met with in other diseases also. Of such diseases, many do not interfere with its diagnostic value because they are scarcely apt to be confounded with

typhoid fever. In others, valuable information may still be obtained if the time of its appearance and disappearance is studied. In typhoid fever it is commonly obtained about the end of the first or the beginning of the second week. It then continues, as a rule, without intermission for a week or ten days, after which it disappears. In my experience a marked reaction is very uncommon after the end of the third or the beginning of the fourth week, and I am inclined to question the diagnosis, if after this the reaction is still marked and if it continues with full intensity.

Microscopic Examination.—Blood corpuscles are not infrequently met with; their occurrence, however, is always an indication that the case is severe. They are most commonly seen in fatal cases, and so in the grave forms which recover, while in the average cases and the benign ones they are rare. They are most commonly met with at the height of the disease. The largest amount of blood is met with in cases of hemorrhagic nephritis. It is to be noted, however, that the amount does not bear any relation to the grade of nephritis, and cases have, indeed, been reported in which, with much blood in the urine during life, the kidneys presented no gross lesions at autopsy (Hewetson). The blood in most cases comes from the kidneys, but it may be of extrarenal origin. In the Hopkins series the hematuria was, as a rule, associated with serous nephritis.

Hemoglobinuria.—Hemoglobinuria is much rarer than hematuria. Osler has reported a case of acute hemorrhagic nephritis complicating typhoid fever, in which hemoglobin alone was present in the urine. Robin also mentions its occurrence.

Casts.—A small number of hyaline and finely granular casts may be seen at any period of the disease. Larger numbers are encountered when the amount of albumin is proportionately larger, and in cases of definite renal involvement all forms may appear. (See Nephritis.)

Pyuria.—Pyuria occurs in a fairly large number of cases (according to Blumer in nearly 17 per cent.). It may appear at any time in the course of the disease, but it is met with most commonly either at the end of the second or in the fourth week. It may persist for a variable length of time and continue well into convalescence. In some cases it persists after the patient is discharged. The amount also is quite variable, but not infrequently large. Sometimes the pus is small in amount at first and then increases; at others the pyuria is marked from the start. Associated with the pus cells are usually casts, epithelial cells, and bacteria. There need, however, be no connection between the pyuria and the cylindruria, and the pyuria may exist alone.

The pus is usually of renal origin; it should be borne in mind, however, that acute cystitis is not so rare in typhoid fever as was formerly supposed. Chronic cystitis of typhoid origin, on the other hand, is rare (Young).

Bacteriuria.—Typhoid bacteriuria occurs in fully 33 per cent. of the cases, and is constant in those which are associated with pyelitis or cystitis. The organism is commonly obtained in pure culture, but in some instances it is found associated with the colon bacillus or staphylococci. It may persist in the urine (as well as the feces and gall-bladder) indefinitely after an attack of the illness (typhoid carriers).

TYPHUS FEVER

Essential Factors.—Irregular leukocytosis with septic factor; presence of the corresponding organism in the blood.

The Blood.—*The Red Corpuscles and Hemoglobin.*—These are probably diminished at the height of the disease; the available data, however, are too meager to warrant any definite conclusions. Hemoglobinemia has been described in some cases.

The Leukocytes.—While hyperleukocytosis is the rule in typhus, normal values or even leukopenia of moderate degree are noted in some cases (20 per cent.). Matthes found values exceeding 10,000 in 34 of 55 cases, and more than 20,000 (up to 25,000) in 3. Of the latter 2 ended fatally and 1 was noted as grave. Usually the values varied between 10,000 and 15,000. The lowest figures were 4000 and 5000. During the active period of the disease the neutrophiles are increased (76 to 88 per cent.) and the eosinophiles absent (septic factor). During convalescence there is a marked tendency to lymphocytosis and maximal or increased eosinophile values (up to 9 per cent.). Emerson gives counts of 4 Hopkins cases, and states that the figures were low or normal on admission, and then rose to a maximum (10,000 to 38,000), which occurred when the temperature had begun to fall or was already normal, after which they fell to normal. His differential counts show the septic factor well pronounced on the days of the maximal counts (neutrophiles up to 93 per cent.). Slatinéano and Galesescu, on the other hand, observed a mononucleosis of the blood in 4 fatal cases.

It is noteworthy that even in those cases which presented no hyperleukocytosis the polynuclear neutrophiles were increased (80 per cent. average) and the eosinophiles absent. The lymphocytes for the most part (60 per cent.) present azurophilic granules (Matthes-Zeller).

Bacteriology.—In both the epidemic (European) and endemic type of the disease the corresponding organism—the Bacillus typhi exanthematosis of Plotz—may be demonstrated in the blood during its earlier period. Positive results seem to be more frequently observed in the epidemic than in the endemic type.

Serology.—Complement fixation may be obtained with a suitable antigen during the postcritical stage of the disease, in a large per-

centage of cases (over 70 per cent.), while before that time only an occasional positive result is observed.

Agglutinins may be demonstrated after the crisis in even a larger percentage of cases than that observed giving complement fixation, viz., over 90 per cent. (Dilutions of 1: 50 or more).

The Cerebrospinal Fluid.—Slatinéano and Galesescu examined the cerebrospinal fluid in 17 cases. It was usually clear. In 12 cases the polynuclear elements outnumbered the mononuclears in the proportion of 5 to 2; in 4 others, all of which ended fatally, the mononuclears predominated, and in these the blood also showed a mononucleosis (*vide supra*).

The Urine.—Special data concerning the condition of the urine are not available.

ULCER (GASTRIC AND DUODENAL)

Essential Factors.—Tendency to chlorotic anemia; normal leukocytosis in uncomplicated cases; hyperleukocytosis in connection with abundant hemorrhage and perforation; septic factor in the latter event, irrespective of the total leukocyte count.

The Blood.—*The Red Cells and Hemoglobin.*—Aside from the occurrence of hemorrhages, there is a distinct tendency to anemia in a fairly large percentage of cases. This affects both the red cells and the hemoglobin, but, as a general rule, the oligochromemia exceeds the oligocythemia, so that low color indices are the outcome. The condition is thus closely comparable to what is seen in uncomplicated chlorosis. In other cases there is no manifest anemia, and in still others, polycythemia may be noted, which is referable, no doubt, to a concentration of the blood in consequence of copious vomiting. In hemorrhagic cases very low counts may be observed. Cabot states that there is no single disease in which the red cells are apt to be so low, with the exception of pernicious anemia. Osler mentions an instance of this kind (duodenal ulcer) where the red cells had fallen to 700,000. In Futcher's series of 44 cases, the lowest count was 1,012,000 and the highest, 4,071,000; while in his series of 88 cases the hemoglobin varied between 12 and 105 per cent., with 58 as average. In Greenough and Joslin's series of 73 cases the color index ranged between 0.35 and 1.41, with 0.67 as average.

In markedly anemic cases, and especially after a copious hemorrhage, there may be a few normoblasts.

The Leukocytes.—In uncomplicated cases the number of leukocytes is normal, and during periods of fasting or feeding by rectum it is often diminished. When food is taken again by mouth there may be a considerable degree of digestive hyperleukocytosis. In one of Cabot's cases the number rose from 4000 to 15,000. Moderate hemorrhages do not necessarily raise the leukocyte count; after copious

bleeding, however, hyperleukocytosis is probably the rule. In one of Howard's cases the number rose to 40,000. Perforation similarly gives rise to hyperleukocytosis, which may only be temporary, however; and at times the individual is apparently overwhelmed with the "toxemia" from the start, so that the count does not exceed the normal, or may actually be subnormal. In cases of this kind the differential count is most important, as the increase of neutrophiles and decrease or absence of eosinophiles which is observed irrespective of the total number, sufficiently indicates the existence of a complicating factor. In hemorrhages the same is seen. In uncomplicated cases the differential count gives normal results.

The Gastric Contents.—Vomiting is a very common symptom, occurring in about 85 per cent. of the cases, especially when the disease is located at the pylorus. It usually takes place within one to three hours after a meal. In complicated cases with dilatation the vomiting may be delayed, and when continuous hypersecretion exists it may occur late at night or in the morning early before any food has been taken. Ordinarily the material represents the constituents of the last meal in various stages of digestion, but in the case last mentioned it is pure gastric juice without any food remnants.

Bleeding from the stomach is very common in gastric ulcer; more common probably than the frequency of vomiting of blood would indicate. The latter occurs in about 75 per cent. of the cases. In some of the remainder no doubt careful and frequently repeated examination of the feces would show that slight bleeding is more common than is generally supposed. Even when a free hemorrhage takes place it may happen that there is no vomiting, the blood being all passed into the intestine. This is more likely to occur if the bleeding is not too abrupt; otherwise the rapid distention of the stomach usually excites vomiting. The appearance of the blood differs in different cases. In about one-third it is bright red. In the rest the color varies according to the duration of its exposure to the hydrochloric acid of the gastric juice, from a reddish brown to brownish black. In such an event one may find no red cells whatever, but in their place amorphous brownish pigment, the nature of which can only be established by chemical examination (which see). Important from a diagnostic standpoint is the fact that the bleeding in gastric ulcer is intermittent and irregular.

The *amount of stomach contents* which may be obtained after Ewald's test breakfast is often somewhat larger than normal (50 c.c. or more), but on the whole the motility of the organ is good.

The *total acidity*, contrary to what is generally supposed, is increased in only about one-third of the cases. Of Friedenwald's 810 cases only 30 per cent. thus showed excessive values of hydrochloric acid, while in 46.6 per cent. the figures were normal and in 23.2 per cent. subnormal. In Howard's series at the Johns Hopkins Hospital

the corresponding values were 27.5, 30, and 42.5 per cent. In Riegel's series of 75 cases the average total acidity was 105. In Howard's series hyperchlorhydria was noted in only 17.6 per cent., normal chlorhydria is 26.4 per cent., and subnormal values in the same proportion, while in 18 free hydrochloric acid was absent. Riegel gives 89 as the maximal figure for free hydrochloric acid with a total acidity of 130. His average value was about 50. Friedenwald has drawn attention to the fact that hyperacidity is proportionately more frequent in males and subacidity in females. According to the same writer the acidity is apt to be very high in acute ulcers and especially in those accompanied by recent hemorrhages, while in chronic cases the values are low.

When carcinoma develops on the basis of an old ulcer it is noteworthy that the secretion of hydrochloric acid—frequently in increased amount—continues, and in rapidly progressing cases may persist to the fatal end. In other cases the acid gradually diminishes and ultimately disappears; such a decline in acidity, when it occurs in an undoubted case of ulcer, should always be viewed with suspicion, particularly if a rapidly growing tumor is palpable at the same time. Sometimes hydrochloric acid may be demonstrated on one day and lactic acid on another.

Riegel found lactic acid present in 6 of 63 cases (14 per cent.), and a doubtful reaction is recorded in 7 per cent. Boas-Oppler bacilli were supposedly seen in 4 of 33 cases. Regarding these findings it is to be noted that lavage was not practised in all the cases previous to the administration of the test meal, which may account for the relatively frequent occurrence of lactic acid.

In *duodenal* ulcer the findings are essentially the same as in the gastric variety; hemorrhage into the stomach, however, is less common, the blood being usually passed in the stools. In Friedenwald's series of 529 cases an examination for occult blood was made in 381 and of these 315 presented one or more positive results.

The Feces.—When free bleeding has occurred in gastric or duodenal ulcer the stools are tarry in appearance, owing to the presence of decomposed blood. Only after exceptionally severe hemorrhages is the natural color of the blood retained, and then only in part. In most cases the microscopic examination shows no well preserved corpuscles, and in cases where the bleeding has been slight the macroscopic appearance is not in the least suggestive of blood. In doubtful cases it is hence necessary to resort to chemical tests for "occult" blood. In this manner much information of value can at times be obtained. In contradistinction to cancer, the blood is *usually* found intermittently in gastric and duodenal ulcer. This rule, however, is not an absolute one, for at times one finds the reaction continuously, until it gradually disappears as the patient recovers. Other sources of hemorrhage must, of course, be excluded before

the presence of blood in the feces can be referred to gastric or duodenal ulcer. Frequently this is easy, but in some cases (cirrhosis of the liver, tubercular enteritis, etc.) it may be difficult.

In Friedenwald's series of 1000 cases the stools were examined in 539 and in 467 of these one or more positive results were obtained (86.6 per cent.), though not always (it should be borne in mind) at the time of the first examination.

The Urine.—When vomiting occurs to any extent and liquids are taken in diminished amount, the total bulk of the urine is naturally diminished. The acidity which even normally is lower after meals is frequently still further decreased when hyperchlorhydria with much vomiting occurs; an alkaline reaction may even result. The chlorides are often diminished when marked hyperchlorhydria exists in association with poor absorption, but the same is true in pyloric obstruction from other causes (carcinoma). Indican contrary, to what one would expect, is frequently much increased, notwithstanding the abundant secretion of hydrochloric acid. The ammonia and nitrogen is said to be usually quite high (8 to 12 per cent. of the total). The total nitrogen may be much reduced in consequence of deficient ingestion (5.2 to 5.9 grams, v. Noorden). The phosphoric acid content is absolutely diminished, owing to defective nutrition (average, 0.7 to 1.3 gram), while the ratio of P_2O_5 to N is on an average 1.67. The uric acid content is normal (0.598 to 0.638, v. Noorden).

Albumin and casts were present in 15 of Howard's series of 77 cases; a trace was noted in 7. Larger amounts may be met with, especially after severe attacks of pain and copious hemorrhages.

UREMIA

Essential Factors.—Increased urea content of the blood; delayed or suspended elimination of phenolsulphonephthalein in the permeation test.

The Blood.—Uremia in itself influences the morphological blood picture only in so far as the question of leukocytosis is concerned. As I have pointed out in the section on chronic nephritis, the average leukocyte count is somewhat higher in the uremic than in the non-uremic cases, but the percentage of cases showing hyperleukocytosis is scarcely as large. (See Chronic Diffuse Nephritis.)

Chemical examination of the blood frequently shows the presence of an unusually large amount of urea, and in former years the uremic complex was attributed to this factor. Subsequently it was shown, however, that this increase is inconstant, and that there may even be a diminished urea content, on the one hand, while, on the other, non-uremic cases may show an excess. The same applies to the content of potassium salts, of the various extractives (notably

kreatinin), and of ammonia, all of which have been similarly held responsible for the uremic symptoms.

The blood examination thus furnishes no factors which can be utilized in the direct diagnosis of the condition.

The Urine.—The same may be said of the urinary picture. The findings are here essentially those of the underlying nephritis (which see). In many cases there is a lowered elimination of urea, but the average figure is practically the same as in the non-uremic cases. Single examinations are of little value, while sudden changes in a curve have much more significance. In the Hopkins series of 96 cases of nephritis with uremia (mentioned by Emerson) there were 13 in which the percentage values[1] were less than 1 per cent.; the average was 0.74 per cent. and the lowest zero. In 21 other cases at the onset of the convulsions the values ranged between 0.9 and 3 per cent., with 1.4 as average. In 123 cases without uremia 1 per cent. or less was occasionally noted in 33 per cent. In 18 fatal cases the average was 1.4., *i. e.*, the same as in the uremic cases (with variations between 0.3 and 3 per cent.).

When improvement occurs there is usually a sharp rise.

The albuminuria and cylindruria are of no interest in the diagnosis of uremia.

The most important factor in the diagnosis of uremia is the greatly delayed or even suspended elimination of phenolsulphonephthalein in the permeation test (see Nephritis).

VACCINATION

The Blood.—The blood picture shows a hyperleukocytosis of the neutrophilic type, amounting to about 15,000, the maximum coinciding in point of time with the maturation of the pustules. A secondary increase has been described as occurring on the tenth or twelfth day; this continues for several days and depends in degree upon the intensity of the local reaction.

VARICELLA

(Chickenpox)

The Blood.—The data regarding the blood picture in varicella are very conflicting. The *red count* and *hemoglobin* values are apparently uninfluenced in uncomplicated cases, and the same, no doubt, holds good for the *leukocytes*. Differing results are probably referable to complicating conditions. When active suppuration occurs the leukocytes will probably be found increased to a greater or less degree, the increase being of the neutrophilic type, with diminution or absence

[1] Percentage values are usually the only ones which can be obtained in such cases.

of the eosinophiles. Nobecourt and Merklen speak of a lymphocytosis and myelocytosis in some of the cases, while others maintain that this does not occur.

The Urine.—The urine usually shows no material deviation from the normal; exceptionally a mild nephritis may be observed.

VARIOLA

(Smallpox)

Essential Factors.—Secondary anemia; irregular hyperleukocytosis, with general tendency to large mononucleosis and lymphocytosis; albuminuria in severe cases.

The Blood.—*The Red Cells and Hemoglobin.*—A certain degree of blood destruction probably occurs in all cases of smallpox, but may be temporarily obscured during the febrile period owing to blood concentration. Subsequently it becomes more manifest and may be quite severe, especially so in the hemorrhagic and confluent cases, in which a count of 2,000,000 to 3,000,000 is not at all uncommon. The anemia may then continue far into convalescence.

The Leukocytes.—Hyperleukocytosis is observed only in severe cases and when pustulation occurs; in the milder forms no increase occurs. In Roger's series of 36 cases there was a count lower than 15,000 (as low as 6000) in 19; in 12 it ranged between 15,000 and 20,000, in 3 between 20,000 and 30,000, and in 2 between 30,000 and 35,000. Higher values, other things being equal, are met with in the non-vaccinated than the vaccinated. The differential findings will depend upon the existence or absence of associated infections. In uncomplicated cases there is a distinct tendency to lymphocytosis and large mononucleosis, while neutrophilic hyperleukocytosis is noted in the secondarily infected cases. Myelocytosis is apparently a frequent event, and in hemorrhagic cases there is said to be an increase of the eosinophiles. The mast cells curiously persist, even in cases showing a marked hyperleukocytosis.

The Plaques.—The plaques are greatly diminished during the febrile period of the disease, and during the stage of pustulation there is a material increase in the tendency to *fibrin* formation.

Parasitology.—Specific organisms have not been satisfactorily demonstrated in the blood. Future research will have to decide the validity of the claim of Councilman, Magrath, and Brinkerhoff that a protozoan parasite, the Cytoryctes variolæ, can be demonstrated in the epithelial cells of the affected areas.

In septic cases *streptococci* and *staphylococci* have been found in the blood.

The Urine.—Albuminuria is a common event in the severer cases, while actual nephritis is rare. In the hemorrhagic cases there may be hematuria.

VARIOLOID

Anemia and hyperleukocytosis are only observed when there is marked suppuration; otherwise the blood picture is normal.

VERRUGA PERUVIANA

The Blood.—In uncomplicated cases of verruga peruviana there may be no anemia of note, but as many of the patients have previously also suffered from malaria, it is more common to find a decrease in the number of the red cells corresponding to the amount of hemoglobin, than normal values. In severe cases the Harvard expedition found red counts ranging between 1,000,000 and 3,900,000 and hemoglobin values between 45 to 75 per cent.

The leukocyte counts in the same series ranged between 4200 and 22,600. Differential counts are reported from two severe advanced cases. In these the small monos ranged between 27 and 39 per cent., the large between 13 and 23, and the polynuclear neutrophiles between 45 and 47 per cent. In one case the eosinophiles numbered 3 per cent., while in the other two cells were seen (*sic.*).

VINCENT'S ANGINA

In cases of Vincent's angina (ulceromembranous angina and stomatitis) smears from the exudate will be seen to contain innumerable organisms which are essentially of two types, viz., spirilla and long, fusiform bacilli (Fig. 166). Occasionally, though exceptionally, the bacilli only may be found. The spirilla usually present three or four convolutions and are generally actively motile. They measure from 36 to 40 μ in length by 0.5 μ in breadth. The bacilli measure from 6 to 12 μ in length, and are somewhat stouter in the middle than at the ends. They may occur in twos, joined end to end, and are usually scattered uniformly throughout the preparation. They are non-motile. Spirilla and bacilli are readily stained with a dilute solution of carbol fuchsin (1 to 20), which should be filtered before use. Löffler's blue and gentian-aniline water may likewise be used.

The bacilli are obligate anaërobes; the spirilla may be obtained together with the bacilli in mixed cultures.

Of late the opinion has been expressed that the spirilla and bacilli may represent stages in the life history of a trypanosome.

Both organisms have occasionally been found associated with diphtheria bacilli.

The disease seems to be more common than was first thought. The earlier cases were reported by Vincent, Bernheim, Conrad, and others. In the United States the disease has been described by Mayer, Fisher, Crandall, Weaver and Tunnicliff, Berkeley and others.

WHOOPING COUGH

Essential Factors.—Hyperleukocytosis; lymphocytosis; iodophilia; presence of the Bacillus pertussis in the sputum.

The Blood.—*The Red Cells and Hemoglobin.*—The red corpuscles and hemoglobin are not materially affected in uncomplicated cases of whooping cough.

The Leukocytes.—According to the researches of Fröhlich and Mennier, and of De Amicis and Pacchioni, hyperleukocytosis is very common in whooping cough during the convulsive stage, the average being about 22,800, with maximal values reaching as high as 51,000. Wanstall, on the other hand, reports that the number is not necessarily increased and that in some cases leukopenia may occur. All observers, however, agree that lymphocytosis (at least relative) is a constant feature of the disease and may be demonstrated already during the catarrhal stage, the number varying between 40 and 60 per cent.; it continues far into convalescence. Wanstall emphasizes that this increase in the number of the lymphocytes is a valuable aid in the diagnosis of whooping cough before the characteristic symptoms of the disease have appeared. It certainly *may* be so; but I would recall the readiness with which small children react with lymphocytosis to various pathological conditions which have nothing to do with whooping cough, and especially to certain winter infections (influenza) in which catarrhal symptoms may also play a prominent role. I am willing to admit, however, that the diagnosis is rendered highly probable if the total number of the leukocytes is simultaneously increased.

In the event of complications the number may be still further increased. Adequate bacteriological examinations are, unfortunately, not available in most cases of this kind in which the blood findings have been recorded, so that it is difficult to account satisfactorily for some of the divergent results which have been reached. Much, no doubt, depends upon the microörganisms which are involved. Personally, I am confident that future studies will show that the lymphocytosis which is so often seen in pertussis pneumonia is not referable to the pneumococcus. Remarkable counts have been obtained in some cases of this order. Cabot mentions two with 69 and 65 per cent. of lymphocytes, in which the total count rose to 94,000, and Strauss cites one with a leukocytosis of 236,000 and 65 per cent. of small mononuclears.

Iodophilia occurs in most of the cases (Crisalfi).

According to Barach an *eosinophilia* is noted, while the leukocytosis and the lymphocytosis fall by lysis, and may continue for a variable time. Usually this is quite moderate, but sometimes it amounts to 10 to 15 per cent.

The Bacteriological Examination.—The bacteriological examination of the blood is negative.

The Sputum.—The sputum, during the catarrhal stage, presents no special characteristics. During the convulsive stage the amount expectorated at one time is very little, but the quantity of twenty-four hours may be quite considerable. At this time it contains bacilli in almost pure culture, which closely resemble the influenza bacillus in appearance and have been described by Spengler, Jochmann Krause, Wallstein, Bordet, and Gengou as the probable causative agent of the disease. The organism is now generally termed the *Bacillus pertussis*, Eppendorf (which see). Wollstein obtained agglutination of the bacillus in question with the serum of the corresponding child, in dilutions of 1 to 200 and occasionally of 1 to 500, and Bordet and Gengou obtained complement fixation with the organism in question and the patient's serum.

The Urine.—The urine of whooping cough presents no special abnormalities in uncomplicated cases. Glucosuria, however, is occasionally observed (Crisalfi).

YAWS

(See FRAMBESIA)

YELLOW FEVER

Essential Factors.—Oligochromemia; no material loss of red cells; irregular neutrophilic hyperleukocytosis; decrease in the specific gravity without corresponding loss of hemoglobin; hemoglobinemia; hematemesis; albuminuria.

The Blood.—*The Red Cells and Hemoglobin.*—According to Pothier and Sternberg, the red count (owing to blood concentration, no doubt) is little if at all affected in yellow fever, while the hemoglobin values are more or less diminished. In Pothier's series of 154 cases the latter ranged between 50 and 90 per cent. during the febrile period, and between 64 and 80 per cent. during convalescence. Exceptionally an isolated normoblast may be found.

Hemoglobinemia has been observed in many cases, and may appear already on the third or fourth day.

The Leukocytes.—The leukocyte count is subject to considerable variation. In Pothier's series it ranged between 4660 and 20,000, and in 5 cases mentioned by Guiteras, between 3200 and 11,400. When hyperleukocytosis exists it is of the neutrophilic type, with high values (85 to 90 per cent.); a few myelocytes may then also be found.

The *specific gravity* of the blood is frequently diminished, even though there is no corresponding loss of hemoglobin (Albertini).

The *etiological factor* of yellow fever is still unknown.

The Stomach Contents.—The vomiting in yellow fever is of the projectile type and noted already in the first stage of the disease. During the third stage, when bleeding commonly occurs, the so-called "black vomit" is observed. The color is referable to the destruction of blood pigment by the acid gastric juice. On microscopic examination the material is seen to contain red blood corpuscles in various stages of destruction, pigment granules, degenerated epithelial cells, leukocytes, and granular detritus. The amount which is vomited at a time varies from a few cubic centimeters to a quart or more.

The Urine.—Albuminuria is observed in nearly all cases, and constitutes an important factor in the diagnosis of the disease; exceptionally, however, it may be absent or it may appear only after the fever has subsided. Usually it is demonstrable on the third or fourth day, and in mild cases it may be found only on these days. In very severe cases it may appear already on the first day. The amount is usually small. Granular and hyaline casts are found in variable number. From the third day on the urine becomes increasingly icteric. The amount is always much reduced, and not infrequently anuria develops.

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